West Updated Search

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, December 13, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	dam.ti,ab,clm. and methyltransferase	16
☐	L2	dam.clm.	3959
☐	L3	L2 and aden$.clm.	12
☐	L4	L3 not l1	4
☐	L5	dam.clm.	3959
☐	L6	L5 and (coli or salmonella or typhimurium or vibrio or yersinia or escherichia or shigella or haemophilus or hemophilus or bordetella or neisseria or pasteruella or treponema).clm.	20
☐	L7	L6 not l1 not l4	9
☐	L8	L7 not l3	9
☐	L9	('6383496')!.PN.	2
☐	L10	dam.ti.	8544
☐	L11	L10 and salmonella	1
☐	L12	DNA near2 aden$ near3 $methylase	71
		DB=USPT; PLUR=YES; OP=AND	
☐	L13	DNA near2 aden$ near3 $methylase	34
☐	L14	dam near5 gene	73
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L15	dam near5 gene	127
☐	L16	L15 or l14	127
☐	L17	L16 same (coli or salmonella or typhimurium or vibrio or yersinia or escherichia or shigella or haemophilus or hemophilus or bordetella or neisseria or pasteruella or treponema)	88
☐	L18	L17 not l1 not l3 not l4 not l6 not l7 not l9 not l11	80

END OF SEARCH HISTORY

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, December 13, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	dam.ti,ab,clm. and methyltransferase	16
☐	L2	dam.clm.	3959
☐	L3	L2 and aden$.clm.	12
☐	L4	L3 not l1	4
☐	L5	dam.clm.	3959
☐	L6	L5 and (coli or salmonella or typhimurium or vibrio or yersinia or escherichia or shigella or haemophilus or hemophilus or bordetella or neisseria or pasteruella or treponema).clm.	20
☐	L7	L6 not l1 not l4	9
☐	L8	L7 not l3	9
☐	L9	('6383496')!.PN.	2

END OF SEARCH HISTORY

Search Results - Record(s) 1 through 16 of 16 returned.

☐ 1. 20040014083. 24 Feb 03. 22 Jan 04. Detection of heteroduplex polynucleotides using mutant nucleic acid repair enzymes with attenuated catalytic activity. Yuan, Chong-Sheng, et al. 435/6; C12Q001/68.

☐ 2. 20030212455. 11 Mar 03. 13 Nov 03. Identification of in vivo dna binding loci of chromatin proteins using a tethered nucleotide modification enzyme. Van Steensel, Bas, et al. 623/6.6; 424/427 435/6 A61F002/16 C12Q001/68 A61F002/00.

☐ 3. 20030203425. 25 Mar 03. 30 Oct 03. Hybrid glycosylated products and their production and use. Leadlay, Peter Francis, et al. 435/68.1; 435/101 435/193 435/75 C12P021/06 C12P019/60 C12P019/04 C12N009/10.

☐ 4. 20030138414. 30 Dec 02. 24 Jul 03. MODULATORS OF METHYLATION FOR CONTROL OF BACTERIAL VIRULENCE. Xu, Mingxu, et al. 424/94.1; A61K038/43.

☐ 5. 20030129603. 24 Oct 01. 10 Jul 03. Databases of regulatory sequences; methods of making and using same. Wolffe, Alan P., et al. 435/6; 435/270 536/25.4 C12Q001/68 C07H021/04.

☐ 6. 20030003472. 20 Feb 02. 02 Jan 03. Mismatch repair detection. Cox, David R., et al. 435/6; 435/471 435/484 435/488 C12Q001/68 C12N015/74.

☐ 7. 20020086332. 09 Aug 01. 04 Jul 02. Method of reducing bacterial proliferation. Mahan, Michael J., et al. 435/7.1; G01N033/53.

☐ 8. 20020086032. 09 Aug 01. 04 Jul 02. Producing antibodies with attenuated bacteria with altered DNA adenine methylase activity. Mahan, Michael J., et al. 424/200.1; 435/252.3 A61K039/02 C12N001/21.

☐ 9. 20020081603. 27 Apr 01. 27 Jun 02. Databases of regulatory sequences; methods of making and using same. Wolffe, Alan, et al. 435/6; 435/91.2 C12Q001/68 C12P019/34.

☐ 10. 20020081317. 09 Aug 01. 27 Jun 02. Bacteria with altered DNA adenine methylase (DAM) activity and heterologous epitope. Mahan, Michael J., et al. 424/200.1; 435/252.3 435/320.1 A61K039/02 C12N001/21 C12N015/74.

☐ 11. 20020077272. 09 Aug 01. 20 Jun 02. Reducing bacterial virulence. Mahan, Michael J., et al. 514/1; 514/263.4 A61K031/00 A61K031/52.

☐ 12. 20020076417. 09 Aug 01. 20 Jun 02. Attenuated bacteria with altered DNA adenine methylase activity. Mahan, Michael J., et al. 424/200.1; 435/252.3 435/252.33 435/252.35 A61K039/02 C12N001/21.

☐ 13. 20020068068. 09 Aug 01. 06 Jun 02. Method of creating antibodies and compositions used for same. Mahan, Michael J., et al. 424/200.1; 424/257.1 424/258.1 424/261.1 A61K039/108 A61K039/112 A61K039/106 A61K039/02.

☐ 14. 5451519. 28 May 93; 19 Sep 95. Cloning restriction endonuclease genes by modulating methyltransferase activity. Collier; Gordon B., et al. 435/199; 435/193 435/252.33 435/320.1 536/23.2. C12N009/22 C12N015/55.

☐ 15. US20020086332A. Reducing virulence or pathogenicity of bacteria, by contacting bacteria with an agent that alters the native level of DNA methyltransferase activity of bacteria or administering the agent to bacteria. HEITHOFF, D M, et al. G01N033/53.

☐ 16. US20020077272A. Reducing bacterial virulence using an agent that alters the bacteria's native level of DNA methyltransferase activity. HEITHOFF, D M, et al. A61K031/00 A61K031/52.

Generate Collection Print

Terms	Documents
dam.ti,ab,clm. and methyltransferase	16

Search Results - Record(s) 1 through 2 of 2 returned.

L9: Entry 1 of 2 File: USPT May 7, 2002

US-PAT-NO: 6383496
DOCUMENT-IDENTIFIER: US 6383496 B1

TITLE: Recombinant vaccines comprising immunogenic attenuated bacteria having RPOS positive phenotype

DATE-ISSUED: May 7, 2002

US-CL-CURRENT: 424/200.1; 424/258.1, 424/93.2, 435/252.3, 435/252.8, 435/471, 435/897

INT-CL: [07] A61 K 39/02, A61 K 48/00, C12 N 15/74, C12 N 1/21

L9: Entry 2 of 2 File: DWPI May 7, 2002

DERWENT-ACC-NO: 2002-442801
ABSTRACTED-PUB-NO: US 6383496B
COPYRIGHT 2004 DERWENT INFORMATION LTD

TITLE: Producing a carrier bacterium for delivering gene product for vaccination includes recombinant rpoS gene, to increase immunogenicity, and attenuating mutations

INT-CL (IPC): A61 K 39/02, A61 K 48/00, C12 N 1/21, C12 N 15/74
Derwent-CL (DC): B04, D16
CPI Codes: B04-B04C; B04-E01; B04-F10A3; B04-F10A8; B04-G01; B14-G02A; B14-G02D; B14-S03; B14-S11; D05-H04; D05-H07; D05-H11; D05-H12A; D05-H12B2; D05-H17A1;

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Search Results - Record(s) 1 through 9 of 9 returned.

☐ 1. 20040192631. 24 Mar 04. 30 Sep 04. DNA vaccines against tumor growth and methods of use thereof. Xiang, Rong, et al. 514/44; A61K048/00.

☐ 2. 20040120962. 15 Apr 03. 24 Jun 04. Modulation of immune responses to foreign antigens expressed by recombinant attenuated bacterial vectors. Curtiss, Roy III, et al. 424/184.1; A61K039/00 A61K039/38.

☐ 3. 20030031683. 03 May 02. 13 Feb 03. Recombinant vaccines comprising immunogenic attenuated bacteria having RpoS positive phenotype. Curtiss, Roy III, et al. 424/200.1; 424/258.1 424/93.2 435/252.3 435/252.8 435/471 435/897 A61K048/00 A01N063/00 A61K039/02 C12N001/20 A61K039/112 C12N015/74 C12N001/00.

☐ 4. 6383496. 18 May 99; 07 May 02. Recombinant vaccines comprising immunogenic attenuated bacteria having RPOS positive phenotype. Curtiss, III; Roy, et al. 424/200.1; 424/258.1 424/93.2 435/252.3 435/252.8 435/471 435/897. A61K039/02 A61K048/00 C12N015/74 C12N001/21.

☐ 5. 6287825. 17 Sep 99; 11 Sep 01. Methods for reducing the complexity of DNA sequences. Weissman; Sherman, et al. 435/91.2; 435/6 435/91.1 436/94 536/23.1 536/24.3 536/24.33. C12P019/34 C12Q001/68 G01N033/00 C07H021/02 C07H021/04.

☐ 6. 6211789. 21 Jan 00; 03 Apr 01. Method and system for manual entry of data into integrated electronic database for livestock data collection. Oldham; Courtney A., et al. 340/573.3; 119/51.02. G08B023/00.

☐ 7. 6103284. 31 Dec 97; 15 Aug 00. Method of preparing waxed in-shell eggs. Polster; Louis S.. 426/298; 426/614. A23B005/00 A23L001/32.

☐ 8. 4343792. 03 Oct 80; 10 Aug 82. Animal vaccine, against colibacillosis, preparation and use. Gouet; Philippe, et al. 424/242.1; 424/257.1 424/823. A61K039/00 A61K039/108.

☐ 9. 4338298. 04 Apr 80; 06 Jul 82. Vaccine for passive immunization against enteric colibacillosis and method of use. Myers; Lyle L.. 424/242.1; 424/257.1 424/823. A61K039/108 A61K039/116.

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Terms	Documents
L7 not L3	9

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US-PAT-NO: 6632430
DOCUMENT-IDENTIFIER: US 6632430 B2

TITLE: Modulators of methylation for control of bacterial virulence

DATE-ISSUED: October 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Xu; Mingxu	San Diego	CA		
Han; Quinghong	San Diego	CA		
Tan; Yuying	San Diego	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
AntiCancer, Inc.	San Diego	CA			02

APPL-NO: 10/ 334532 [PALM]
DATE FILED: December 30, 2002

PARENT-CASE:
This application is a divisional of U.S. Ser. No. 09/591,078 filed Jun. 9, 2000 abandoned which application claims priority from Ser. No. 60/138,307 filed Jun. 9, 1999. The contents of these applications are incorporated herein by reference.

INT-CL: [07] A61 K 38/51, C12 N 9/00, C12 N 9/88

US-CL-ISSUED: 424/94.5; 435/183, 435/232
US-CL-CURRENT: 424/94.5; 435/183, 435/232

FIELD-OF-SEARCH: 435/183, 435/232, 424/94.5

PRIOR-ART-DISCLOSED:

L13: Entry 26 of 34 File: USPT Mar 24, 1998

DOCUMENT-IDENTIFIER: US 5731185 A
**** See image for Certificate of Correction ****
TITLE: Isolated DNA encoding the hphi restriction endonuclease and related methods for producing the same

Drawing Description Text (18):
FIG. 9 is a diagram of the DNA amplification of the adenine methylase gene.

US-PAT-NO: 4798791
DOCUMENT-IDENTIFIER: US 4798791 A

TITLE: Vector for high level gene expression

DATE-ISSUED: January 17, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Anderson; David M.	Rockville	MD		
McGuire; Jeffrey C.	Frederick	MD		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Genex Corporation	Gaithersburg	MD			02

APPL-NO: 06/ 671967 [PALM]
DATE FILED: November 16, 1984

INT-CL: [04] C12P 21/00, C12P 19/34, C12N 5/00, C12N 7/00

US-CL-ISSUED: 435/68; 435/70, 435/91, 435/172.3, 435/317, 435/320, 935/6, 935/22, 935/29, 935/48, 935/73, 536/27
US-CL-CURRENT: 435/69.1; 435/219, 435/226, 435/317.1, 435/320.1, 435/481, 435/91.41, 536/23.1, 536/24.1

FIELD-OF-SEARCH: 435/68, 435/71, 435/91, 435/253, 435/317, 435/172.3, 536/27, 935/6, 935/29, 935/22, 935/41, 935/48, 935/56, 935/72, 935/40, 935/60, 935/73

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4643969	February 1987	Inouye et al.	435/68
☐	4666848	May 1987	Gelfand et al.	

OTHER PUBLICATIONS

Wu et al., (1981) Proceedings National Academy Sciences, USA, vol. 78, pp. 2913-2917.
Holmes et al. (1983), Cell, vol. 32, pp. 1029-1032.
Platt et al. (1985, Mtg. Held) UCLA Symp. on Molecular and Cellular Biol., New Ser., vol. 30, pp. 151-160.
Schmeissner, U., et al., J. Mol. Biol. 176: 39-53 (1984).

Platt, T., et al., in, R. Calendar and L. Gold, eds., Sequence Specificity in Transcription and Translation, Alan R. Liss, Inc., Publisher, New York, New York (1985).
Holmes et al., Cell, 32:1029-1032 (1983).
Guarente et al., J. Mol. Biol., 133:189-197 (1979).
Chernajovsky et al., Annals of the New York Academy of Science, 413:88-96 (1983).
Platt, T., The Operon, pp. 290-294, Cold Spring Harbor, New York (1978).
Mellor et al., Gene, 24:1-14 (1983).
Wu et al., Cell, 19:829-836 (1980).
Mott, J. E., The American Biology Teacher, 46:388-393 (1984).

ART-UNIT: 185

PRIMARY-EXAMINER: Wiseman; Thomas G.

ASSISTANT-EXAMINER: Seidman; S.

ATTY-AGENT-FIRM: Saidman, Sterne, Kessler & Goldstein

ABSTRACT:

Methods and vectors for high level expression of genes in bacteria are disclosed. A terminal mRNA sequence from a gene coding for a stable bacterial protein mRNA is ligated to a gene coding for the desired protein adjacent the translation termination codon of the gene. The gene for the desired protein and the terminal mRNA sequence are situated in an expression vector in which the gene is operably linked to a transcription promoter.

23 Claims, 2 Drawing figures

promoter 5126 and gene to achieve male sterility. Cigan; Andrew M., et al. 800/298; 435/320.1 435/424 435/430 47/DIG.1 536/24.1 800/303. C12H015/00 C12H015/05 A01H001/06 A01H004/00.

☐ 28. 5689049. 07 Jun 95; 18 Nov 97. Transgenic plant and method for producing male sterility using anther specific promoter 5126. Cigan; Andrew M., et al. 800/287; 47/DIG.1 536/24.1 800/303. C12N015/00 C12N015/05 A01H001/06 A01H004/00.

☐ 29. 5624820. 07 Jun 95; 29 Apr 97. Episomal expression vector for human gene therapy. Cooper; Mark J.. 435/69.1; 435/320.1 536/23.72. C12P021/06 C12N015/37 C12N015/85.

☐ 30. 5202256. 16 Aug 91; 13 Apr 93. Bioadhesive precursor protein expression vectors. Maugh; Kathy J., et al. 435/252.3; 435/254.21 435/320.1 435/69.1. C12N001/19 C12N001/21 C12N015/81 C12N015/12.

☐ 31. 5202236. 25 May 90; 13 Apr 93. Method of producing bioadhesive protein. Maugh; Kathy J., et al. 435/69.1; 435/69.7 530/353. C12P021/02 C12P021/06 C12N015/12 C12N015/62.

☐ 32. 5049504. 30 May 90; 17 Sep 91. Bioadhesive coding sequences. Maugh; Kathy J., et al. 435/252.33; 435/254.21 435/320.1 435/69.1 530/350 536/23.5. C12N001/21 C12N015/12 C12N001/19 C12N015/63.

☐ 33. 5034322. 05 Apr 89; 23 Jul 91. Chimeric genes suitable for expression in plant cells. Rogers; Stephen G., et al. 435/252.2; 435/183 435/252.3 435/252.33 435/320.1 435/69.1 536/23.2 536/23.7. C12P021/00 C12N015/00 C12N009/00 C12N001/20.

☐ 34. 4798791. 16 Nov 84; 17 Jan 89. Vector for high level gene expression. Anderson; David M., et al. 435/69.1; 435/219 435/226 435/317.1 435/320.1 435/481 435/91.41 536/23.1 536/24.1. C12P021/00 C12P019/34 C12N005/00 C12N007/00.

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DOCUMENT-IDENTIFIER: US 6632430 B2
TITLE: Modulators of methylation for control of bacterial virulence

Brief Summary Text (4):
The importance of transmethylation reactions in metabolism in general has gained considerable recognition. PCT application WO96/20010 and U.S. Pat. No. 5,872,104, incorporated herein by reference, describe the use of methylation inhibitors to reduce the resistance of microorganisms to antibiotics. Heithoff, D. M., et al,. Science (1999) 284:967-970 report the results of a study showing that Salmonella typhimurium which lacks DNA adenine methylase (Dam) were essentially avirulent and therefore could be used as live vaccines against murine typhoid fever. The authors concluded that Dam regulated the expression of at least 20 genes known to be induced during infection and noted that inhibitors of Dam were likely to be antimicrobials. It was earlier shown by Braaten, B. A., et al,. Cell (1994) 76:577-588 that the methylation patterns associated with pyelonephritis-associated pili (Pap) DNA controlled gene expression in E. coli. Thus, it is clear that in bacteria, methylation status is significant in controlling metabolism, and thus infectivity in general. The importance of S-adenosyl-L-methionine (SAM) dependent transmethylation in viral infection has also been studied by Liu, S., et al., Antiviral Research (1992) 19:247-265.

L18: Entry 31 of 80 File: USPT Jul 23, 2002

DOCUMENT-IDENTIFIER: US RE37806 E
TITLE: Method of using eukaryotic expression vectors comprising the BK virus enhancer

Detailed Description Text (252):
To construct plasmid phd, it was necessary to prepare the plasmid pLPChd1 DNA, used as starting material in the construction of plasmid phd, from E. coli host cells that lack an adenine methylase, such as that encoded by the dam gene, the product of which methylates the adenine residue in the sequence 5'-GATC-3' E. coli K12 GM48 (NRRL B-15725) lacks a functional dam methylase and so is a suitable host to use for the purpose of preparing plasmid pLPChd1 DNA for use as starting material in the construction of plasmid phd.

transgenic plants. Cigan; Andrew M., et al. 800/267; 536/23.6 536/23.7 536/24.1 800/266 800/271 800/274. C12N001/00 A01H005/00 A01H005/04.

☐ 15. 6174724. 04 May 95; 16 Jan 01. Chimeric genes suitable for expression in plant cells. Rogers; Stephen G., et al. 435/419; 435/252.2 435/252.3 435/252.33 435/418 435/469 435/69.1 435/70.1 536/23.2 536/23.6 536/23.7 536/24.1 800/288 800/294. C12N015/82 C12N015/84 C12N005/04 C12N015/31.

☐ 16. 6147278. 03 Mar 99; 14 Nov 00. Plant vectors. Rogers; Stephen G., et al. 800/278; 435/320.1 435/468 435/469 435/69.1 536/23.72 800/288. C12N015/34 C12N015/82 C12N015/83 C12N015/84.

☐ 17. 6096526. 20 May 98; 01 Aug 00. Human nucleic acid methylases. Hillman; Jennifer L., et al. 435/193; 435/252.3 435/320.1 435/6 536/23.2. C12N009/10 C12N015/00 C12N001/20 C12Q001/68 C07H021/04.

☐ 18. 6072102. 28 Nov 97; 06 Jun 00. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 800/274; 536/24.1 800/271 800/275 800/285 800/288. A01H001/00 C12M015/00.

☐ 19. 6030956. 23 Oct 97; 29 Feb 00. Combination gene therapy for human cancers. Boulikas; Teni. 514/44; 428/402.2 435/320.1 536/23.2 536/23.5 536/24.1. C12N015/00 A01N043/04 C07H021/04 B32B005/16.

☐ 20. 5837851. 07 Jun 95; 17 Nov 98. DNA promoter 5126 and constructs useful in a reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 536/24.1; 536/23.1 536/27.1. C07H021/04 C12N015/63.

☐ 21. 5795753. 07 Jun 95; 18 Aug 98. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 800/274; 536/24.1 800/275 800/287 800/293 800/303 800/320.1. C12N015/00 A01H001/06 A01H005/00.

☐ 22. 5792853. 07 Jun 95; 11 Aug 98. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 536/24.1; 47/DIG.1 536/23.1 536/23.6 800/303. C07H021/02 C07H021/04 C12N015/00 C12N015/05.

☐ 23. 5770374. 10 Oct 96; 23 Jun 98. Methods for identifying oncogenes and anti-oncogenes. Cooper; Mark J.. 435/6;. C12Q001/68.

☐ 24. 5763243. 07 Jun 95; 09 Jun 98. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 800/267; 47/DIG.1 536/24.1 536/27.1 800/274 800/287. C12N015/00 A01H004/00 A01H001/00.

☐ 25. 5750868. 08 Dec 94; 12 May 98. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 800/274; 435/424 435/430 47/DIG.1 536/24.1 536/27.1 800/287 800/303. C12H015/00 C12H015/05 A01H001/06 A01H004/00.

☐ 26. 5731185. 21 Jul 95; 24 Mar 98. Isolated DNA encoding the hphi restriction endonuclease and related methods for producing the same. Meda; Marta M., et al. 435/194; 435/252.3 435/320.1 536/23.1. C12N009/22 C12N015/55.

☐ 27. 5689051. 07 Jun 95; 18 Nov 97. Transgenic plants and DNA comprising anther specific

☐ 1. 6673538. 21 Apr 00; 06 Jan 04. Methods and compositions for designing vaccines. Goldstein; Richard N.. 435/6; 435/243. C12Q001/68 C12N007/00.

☐ 2. 6632430. 30 Dec 02; 14 Oct 03. Modulators of methylation for control of bacterial virulence. Xu; Mingxu, et al. 424/94.5; 435/183 435/232. A61K038/51 C12N009/00 C12N009/88.

☐ 3. 6610489. 27 Apr 01; 26 Aug 03. Pharmacogenomics and identification of drug targets by reconstruction of signal transduction pathways based on sequences of accessible regions. Wolffe; Alan, et al. 435/6; 435/7.1. C12Q001/68 G01N033/00.

☐ 4. 6566107. 01 Nov 00; 20 May 03. Recombinant Zymomonas mobilis with improved xylose utilization. Zhang; Min. 435/161; 435/194 435/233 435/243 435/252.3 435/41 435/455 435/61. C12P007/06.

☐ 5. 6528289. 23 Aug 00; 04 Mar 03. Nucleotide sequence of the Haemophilus influenzae Rd genome, fragments thereof, and uses thereof. Fleischmann; Robert D., et al. 435/91.41; 435/252.3 435/320.1 435/6 536/23.1 536/23.7. C12N015/64.

☐ 6. 6511808. 27 Apr 01; 28 Jan 03. Methods for designing exogenous regulatory molecules. Wolffe; Alan, et al. 435/6;. C12Q001/68.

☐ 7. 6506581. 25 Apr 00; 14 Jan 03. Nucleotide sequence of the Haemophilus influenzae Rd genome, fragments thereof, and uses thereof. Fleischmann; Robert D., et al. 435/69.1; 435/252.3 435/320.1 435/69.3 435/91.41 536/23.7. C12N001/21 C12N015/31 C12N015/63.

☐ 8. 6495346. 12 Jan 00; 17 Dec 02. Complex-forming proteins. Jerome; Valerie, et al. 435/69.7; 424/85.1 424/85.2 435/69.5 435/69.52 530/351 536/23.4 536/23.5 536/23.51. C12N015/62 A61K038/20 C07K014/54.

☐ 9. 6436683. 18 May 00; 20 Aug 02. Human nucleic acid methylases. Hillman; Jennifer L., et al. 435/193; 435/25 435/252.3 435/320.1 435/325 435/440 536/23.2. C12N009/10 C12N001/20 C12N015/00 C12Q001/26 C07H021/04.

☐ 10. 6399856. 19 Nov 98; 04 Jun 02. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 800/274; 536/24.1 800/275 800/278. A01H001/00 A01H001/022 A01H015/82.

☐ 11. 6355450. 07 Jun 95; 12 Mar 02. Computer readable genomic sequence of Haemophilus influenzae Rd, fragments thereof, and uses thereof. Fleischmann; Robert D., et al. 435/69.1; 435/252.3 435/320.1 435/851 536/23.1 536/23.7 536/24.32 536/24.33. C12P021/06 C12N001/20 C12N015/00 C07H021/04.

☐ 12. 6339065. 30 Jan 96; 15 Jan 02. Episomal expression vector for human gene therapy. Cooper; Mark J.. 514/44; 435/455 435/69.1. A61K031/70 C12N015/85 C12P021/02.

☐ 13. 6281348. 17 Mar 97; 28 Aug 01. Reversible nuclear genetic system for male sterility in transgenic plants. Cigan; Andrew M., et al. 536/24.1; 536/23.1 536/23.6 800/271 800/272 800/303. C12N015/00 C12N015/05 C07H021/02 C07H021/04.

☐ 14. 6248935. 28 Nov 97; 19 Jun 01. Reversible nuclear genetic system for male sterility in

L18: Entry 68 of 80 File: USPT Apr 9, 1996

DOCUMENT-IDENTIFIER: US 5506118 A

**** See image for Certificate of Correction ****

TITLE: Method of using eukaryotic expression vectors comprising a poly GT element in the presence of trans-acting gene products

Detailed Description Text (255):

To construct plasmid phd, it was necessary to prepare the plasmid pLPChd1 DNA (Example 12), used as starting material in the construction of plasmid phd, from E. coli host cells that lack an adenine methylase, such as that encoded by the dam gene, the product of which methylares the adenine residue in the sequence 5'-GATC-3'. E. coli K12 GM48 (NRRL B-15725) lacks a functional dam methylase and so is a suitable host to use for the purpose of preparing plasmid pLPChd1 DNA for use as starting material in the construction of plasmid phd.

L18: Entry 69 of 80 File: USPT Oct 24, 1995

DOCUMENT-IDENTIFIER: US 5460953 A
**** See image for Certificate of Correction ****
TITLE: Vectors and compounds for expression of glycosylation mutants of human protein C

Detailed Description Text (63):
Plasmid pGTC is one such vector, wherein the wild type human protein C zymogen gene is driven by the GBMT transcription unit. The wild type protein C gene can be easily removed from the vector on a BclI restriction fragment and any of the genes of the present invention can be inserted into the vector on a BclI restriction fragment. Digestion of plasmid DNA with BclI is inhibited by methylation at adenine in the sequence 5'-GATC-3'. Therefore, plasmid pGTC was prepared from E. coli host cells that lack an adenine methylase, such as that encoded by the dam gene, the product of which methylates the adenine residue in the sequence 5'-GATC-3'. E. coli K12 GM48 (NRRL B-15725) lacks a functional dam methylase and so is a suitable host to use for the purpose of preparing plasmid pGTC DNA for use as starting material in the construction of derivative plasmids.

L18: Entry 70 of 80 File: USPT Feb 21, 1995

DOCUMENT-IDENTIFIER: US 5391712 A
TITLE: Non-hemolytic streptolysin O variants

Detailed Description Text (54):
The dam, mutS, mutR, mutL, and uvrD genes are involved in the process of mismatch repair; accordingly, E. coli strains which include defective versions of these genes genes exhibit moderately high mutator activity. Some E. coli mutator genes cause specific changes in the DNA sequence being replicated. For example, the mutS3 mutator strain of E. coli produces bi-directional transitions and is apparently sensitive to base sequence, in that it mutates one A:T base pair but does not mutate a second A:T base pair located less than 50 nucleotides away. The mutt mutator strain of E. coli is unique in displaying a strict specificity, i.e., only A:T.fwdarw.C:G transitions are induced. The mutD mutator strain of E. coli is also notable because it results in mutation frequencies of 10.sup.3 to 10.sup.5 times that of wild-type E. coli. Transversions of A:T.rarw..fwdarw.G:T, transitions of A:T.rarw..fwdarw.T:A, and A:T.fwdarw.C:G substitutions are associated with the mutD strain. While the tendencies of certain mutator strains are known, predicting exactly when or where along the DNA macromolecule a mutation will take place cannot be reasonably predicted.

L18: Entry 71 of 80 File: USPT Jan 17, 1995

DOCUMENT-IDENTIFIER: US 5382429 A
TITLE: Bacillus thuringiensis protein toxic to coleopteran insects

Other Reference Publication (23):
Marinus et al., Mol. Gen. Genet., 192, pp. 288-289 (1983) "Insertion Mutations in the dam gene of Escherichia coli K-12".

```
        13dec04 15:19:40 User228206 Session D2305.1
             $0.00    0.207 DialUnits FileHomeBase
      $0.00  Estimated cost FileHomeBase
      $0.00  Estimated cost this search
      $0.00  Estimated total session cost   0.207 DialUnits
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update Dialog 12/04

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File 155:MEDLINE(R) 1951-2004/Dec W1
       (c) format only 2004 The Dialog Corp.
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***File 155: Medline has stopped updating as of December 7, 2004.**

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Please see HELP NEWS 155 for details.

      Set  Items  Description
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?e methylase

Ref   Items  Index-term
E1        2  METHYLASCORBIC
E2        9  METHYLASCORBIGEN
E3     1976 *METHYLASE
E4      934  METHYLASES
E5      532  METHYLASES //DNA MODIFICATION (DNA MODIFICATION METHYLASES)
E6        3  METHYLASIMILOBINE
E7        1  METHYLASPARAGINAMIDE
E8       10  METHYLASPARAGINE
E9        1  METHYLASPARAGINES
E10       2  METHYLASPARATE
E11       1  METHYLASPARIC
E12      25  METHYLASPARTASE

          Enter P or PAGE for more
?s e3 or e4 or e5
            1976  METHYLASE
             934  METHYLASES
             532  METHYLASES //DNA MODIFICATION  (DNA MODIFICATION
                  METHYLASES)
      S1    2641  'METHYLASE' OR 'METHYLASES' OR 'METHYLASES //DNA
                  MODIFICATION'
?e dna adenine methyltransferase

Ref   Items   RT  Index-term
E1        6       DNA ADDUCTS --ULTRASTRUCTURE --UL
E2       36       DNA ADDUCTS --URINE --UR
E3        0      *DNA ADENINE METHYLTRANSFERASE
E4       31       DNA ALKYLTRANSFERASE
E5        0    1  DNA AMPLIFICATION TECHNIQUES
E6        1       DNA B-26
E7        9       DNA BETA-GLUCOSYLTRANSFERASE
E8        0    1  DNA COMPUTERS
E9        0    1  DNA CYTOSINE-5-METHYLASE
E10   23580   11  DNA DAMAGE
E11       1       DNA DAMAGE --CLASSIFICATION --CL
E12    2134       DNA DAMAGE --DRUG EFFECTS --DE

          Enter P or PAGE for more
?e methyltransferase

Ref   Items  Index-term
E1       23  METHYLTRANSFER
E2        1  METHYLTRANSFERAE
E3    13529 *METHYLTRANSFERASE
E4      653  METHYLTRANSFERASE  (ADENINE-S //SITE-SPECIFIC D (SITE-SPECIFIC
             DNA-METHYLTRANSFERASE (ADENINE-S)
E5      583  METHYLTRANSFERASE  (CYTOSINE- //SITE-SPECIFIC D (SITE-SPECIFIC
             DNA METHYLTRANSFERASE (CYTOSINE-)
E6      342  METHYLTRANSFERASE    //ACETYLSEROTONIN    N    (ACETYLSEROTONIN
```

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               N-METHYLTRANSFERASE)
E7      1957   METHYLTRANSFERASE //CATECHOL O (CATECHOL O-METHYLTRANSFERASE)
E8      1107   METHYLTRANSFERASE //DNA (CYTOSINE-5-) (DNA (CYTOSINE-5-)-METH-
               YLTRANSFERASE)
E9       295   METHYLTRANSFERASE //HISTAMINE N (HISTAMINE N-METHYLTRANSFERAS-
               E)
E10      103   METHYLTRANSFERASE     //HISTONE-LYSINE    N    (HISTONE-LYSINE
               N-METHYLTRANSFERASE)
E11     1328   METHYLTRANSFERASE //O(6)-METHYLGUANINE-DNA (O(6)-METHYLGUANIN-
               E-DNA METHYLTRANSFERASE)
E12     1126   METHYLTRANSFERASE  //PHENYLETHANOLAMINE  N (PHENYLETHANOLAMINE
               N-METHYLTRANSFERASE)
          Enter P or PAGE for more
?e gatc

Ref   Items  Index-term
E1        1  GATBONTON
E2        6  GATBP
E3      376 *GATC
E4        4  GATCA
E5        4  GATCAB
E6        1  GATCAT
E7        1  GATCBA
E8       13  GATCC
E9        1  GATCCAAGCAAACACTATTTGTCCTCCTACTA
E10       1  GATCCATATGGCAGGGTA
E11       1  GATCCCCGGG
E12       3  GATCCGCG

          Enter P or PAGE for more
?s e3
      S2     376  'GATC'
?s methyltransferase?
      S3   15734  METHYLTRANSFERASE?
?s s2 and s3
             376  S2
           15734  S3
      S4     148  S2 AND S3
?ds

Set     Items   Description
S1       2641   'METHYLASE' OR 'METHYLASES' OR 'METHYLASES //DNA MODIFICAT-
             ION'
S2        376   'GATC'
S3      15734   METHYLTRANSFERASE?
S4        148   S2 AND S3
?s s1 or s4
            2641  S1
             148  S4
      S5    2728  S1 OR S4
?s s5/1999:2004
            2728  S5
         3082950  PY=1999 : PY=2004
      S6     720  S5/1999:2004
?s s5 not s6
            2728  S5
             720  S6
      S7    2008  S5 NOT S6
?s s7 and (attenuat? or mutant? or mutation? or mutagen? or insert? or delet? or modif?
 or alter?)
            2008  S7
          103231  ATTENUAT?
          201536  MUTANT?
          315477  MUTATION?
          112134  MUTAGEN?
          117579  INSERT?
          130982  DELET?
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        367950  MODIF?
        642706  ALTER?
    S8    1347  S7 AND (ATTENUAT? OR MUTANT? OR MUTATION? OR MUTAGEN? OR
                INSERT? OR DELET? OR MODIF? OR ALTER?)
?s s8 and (secherichia? or vibrio? or yersinia? or salmonella? or neisseria? or bordete
lla? or shigella? or pasteurella? or hemophilus? or haemophilus?)
          1347  S8
             0  SECHERICHIA?
         13981  VIBRIO?
          8570  YERSINIA?
         54809  SALMONELLA?
         16417  NEISSERIA?
         11596  BORDETELLA?
         11141  SHIGELLA?
          6694  PASTEURELLA?
          1648  HEMOPHILUS?
         19109  HAEMOPHILUS?
    S9     102  S8 AND (SECHERICHIA? OR VIBRIO? OR YERSINIA? OR
                SALMONELLA? OR NEISSERIA? OR BORDETELLA? OR SHIGELLA? OR
                PASTEURELLA? OR HEMOPHILUS? OR HAEMOPHILUS?)
?s s8 and (echerichia? or vibrio? or yersinia? or salmonella? or neisseria? or bordetel
la? or shigella? or pasteurella? or hemophilus? or haemophilus?)
          1347  S8
            43  ECHERICHIA?
         13981  VIBRIO?
          8570  YERSINIA?
         54809  SALMONELLA?
         16417  NEISSERIA?
         11596  BORDETELLA?
         11141  SHIGELLA?
          6694  PASTEURELLA?
          1648  HEMOPHILUS?
         19109  HAEMOPHILUS?
   S10     102  S8 AND (ECHERICHIA? OR VIBRIO? OR YERSINIA? OR
                SALMONELLA? OR NEISSERIA? OR BORDETELLA? OR SHIGELLA? OR
                PASTEURELLA? OR HEMOPHILUS? OR HAEMOPHILUS?)
?
?s s10 and gene?
           102  S10
       2322278  GENE?
   S11      83  S10 AND GENE?
?target s11/all
Your TARGET search request will retrieve up to 50 of the statistically most
relevant records.
Searching ALL records
...Processing Complete
   S12      50  TARGET - S11
Ending TARGET search. Enter TARGET to do another search in the present
file(s), or BEGIN new file(s).  Enter LOGOFF to disconnect from Dialog
?t s12/9/all
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12/9/1
DIALOG(R)File 155:MEDLINE(R)
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13324299   PMID: 8996109
Molecular cloning and expression of NlaIII restriction-modification system in E. coli.
Morgan R D; Camp R R; Wilson G G; Xu S Y
New England Biolabs Inc., Beverly, MA 01915, USA.
Gene (NETHERLANDS)   Dec 12 1996,   183 (1-2) p215-8,   ISSN 0378-1119
Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

were purified. A short DNA fragment carrying the HgaI recognition site was treated with each of these enzymes, and, after separation of the two strands by duplex formation with M13 viral DNAs carrying the respective strands, the presence or absence of **modification** was judged from susceptibility to HgaI endonuclease. The results of analysis showed that different strands were **modified** in an asymmetric way by each **gene** product. Analysis of the species and positions of **modified** bases by the Maxam-Gilbert method further demonstrated that the **gene** products from the upstream and middle ORFs participated in methylation of the internal cytosine residues of the strands carrying 3'-CTGCG-5' and 5'-GACGC-3', respectively. We concluded that the HgaI **modification** system consisted of two cytosine **methylase genes** responsible for **modification** of different strands in the target DNA.

Tags: Support, Non-U.S. Gov't

Descriptors: *Genes, Structural, Bacterial; *Haemophilus--enzymology--EN; *Site-Specific DNA Methyltransferase (Cytosine-Specific)--genetics--GE; *Site-Specific DNA Methyltransferase (Cytosine-Specific)--metabolism--ME; Amino Acid Sequence; Base Sequence; Cloning, Molecular; DNA, Bacterial --genetics--GE; DNA, Bacterial--metabolism--ME; Gene Expression Regulation, Bacterial; Haemophilus--genetics--GE; Methylation; Molecular Sequence Data; Restriction Mapping

Molecular Sequence Databank No.: GENBANK/D90363; GENBANK/M63255; GENBANK/M63832; GENBANK/M63833; GENBANK/M63834; GENBANK/M63835; GENBANK/M63926; GENBANK/M63977; GENBANK/M63978; GENBANK/M64288

CAS Registry No.: 0 (DNA, Bacterial)

Enzyme No.: EC 2.1.1.- (DNA modification methylase HgaI); EC 2.1.1.73 (Site-Specific DNA Methyltransferase (Cytosine-Specific))

Record Date Created: 19910823

Record Date Completed: 19910823

12/9/4

DIALOG(R)File 155:MEDLINE(R)

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13880569 PMID: 9575240

A temperature-sensitive DNA adenine methyltransferase mutant of Salmonella typhimurium.

Brawer R; Batista F D; Burrone O R; Sordelli D O; Cerquetti M C

Centro de Estudios Farmacoligicos y Botanicos (CEFYBO-CONICET) and University of Buenos Aires, School of Medicine, Serrano 669, 1414 Buenos Aires, Argentina.

Archives of microbiology (GERMANY) Jun 1998, 169 (6) p530-3, ISSN 0302-8933 Journal Code: 0410427

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A temperature-sensitive mutant of Salmonella typhimurium was isolated earlier after transposon mutagenesis with Tn10d Tet. The mutant D220 grows well at 28 degreesC but has a lower growth rate and forms filaments at 37 degreesC. Transposon-flanking fragments of mutant D220 DNA were cloned and sequenced. The transposon was **inserted** in the dam **gene** between positions 803 and 804 (assigned allele number: dam-231 : : Tn10d Tet) and resulted in a predicted ten-amino-acid-shorter Dam protein. The **insertion** created a stop codon that led to a truncated Dam protein with a temperature-sensitive phenotype. The **insertion** dam-231 : : Tn10d Tet resulted in a dam "leaky" phenotype since methylated and unmethylated adenines in **GATC** sequences were present. In addition, the dam-231 : : Tn10d Tet **insertion** rendered dam **mutants** temperature-sensitive for growth depending upon the **genetic** background of the S. typhimurium strain. The wild-type dam **gene** of S. typhimurium exhibited 82% identity with the Escherichia coli dam **gene** .

Tags: Support, Non-U.S. Gov't

Descriptors: *Salmonella typhimurium--enzymology--EN; *Site-Specific DNA-Methyltransferase (Adenine-Specific)--genetics--GE; DNA Transposable

Elements--genetics--GE; Polymerase Chain Reaction; Salmonella typhimurium --genetics--GE; Salmonella typhimurium--growth and development--GD; Site-Specific DNA-Methyltransferase (Adenine-Specific) --isolation and purification--IP; Temperature
CAS Registry No.: 0 (DNA Transposable Elements)
Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA-Methyltransferase (Adenine-Specific))
Record Date Created: 19980713
Record Date Completed: 19980713

12/9/5
DIALOG(R)File 155:MEDLINE(R)
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08783943 PMID: 1846861
Two-step cloning and expression in Escherichia coli of the DNA restriction-modification system StyLTI of Salmonella typhimurium.
De Backer O; Colson C
Departement de Biologie, Universite Catholique de Louvain, Louvain-la-Neuve, Belgium.
Journal of bacteriology (UNITED STATES) Feb 1991, 173 (3) p1321-7, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The StyLTI restriction-modification system is common to most strains of the genus Salmonella, including Salmonella typhimurium. We report here the two-step cloning of the genes controlling the StyLTI system. The StyLTI methylase gene (mod) was cloned first. Then, the companion endonuclease gene (res) was introduced on a compatible vector. A strain of S. typhimurium sensitive to the coliphage lambda was constructed and used to select self-modifying recombinant phages from a Res- Mod+ S. typhimurium genomic library in the lambda EMBL4 cloning vector. The **methylase gene** of one of these phages was then subcloned in pBR328 and transferred into Escherichia coli. In the second step, the closely linked endonuclease and **methylase genes** were cloned together on a single DNA fragment **inserted** in pACYC184 and introduced into the Mod+ E. coli strain obtained in the first step. Attempts to transform Mod- E. coli or S. typhimurium strains with this Res+ Mod+ plasmid were unsuccessful, whereas transformation of Mod+ strains occurred at a normal frequency. This can be understood if the introduction of the StyLTI **genes** into naive hosts is lethal because of degradation of host DNA by restriction activity; in contrast to most restriction- **modification** systems, StyLTI could not be transferred into naive hosts without killing them. In addition, it was found that strains containing only the res **gene** are viable and lack restriction activity in the absence of the companion mod **gene** . This suggests that expression of the StyLTI endonuclease activity requires at least one polypeptide involved in the methylation activity, as is the case for types I and III restriction- **modification** systems but not for type II systems.
Tags: Support, Non-U.S. Gov't
Descriptors: *DNA Modification Methylases--genetics--GE; *DNA Restriction Enzymes--genetics--GE; *Deoxyribonucleases, Type III Site-Specific --genetics--GE; *Escherichia coli--genetics--GE; *Gene Expression Regulation, Bacterial; *Salmonella typhimurium--genetics--GE; *Site-Specific DNA-Methyltransferase (Adenine-Specific)--genetics--GE; Cloning, Molecular; DNA Modification Methylases--metabolism--ME; DNA Restriction Enzymes--metabolism--ME; Deoxyribonucleases, Type III Site-Specific--metabolism--ME; Genes, Bacterial; Phenotype; Plasmids; Restriction Mapping; Salmonella typhimurium--enzymology--EN; Site-Specific DNA-Methyltransferase (Adenine-Specific)--metabolism--ME; Transformation, Bacterial
CAS Registry No.: 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA Modification Methylases); EC 2.1.1.- (DNA modification methylase StyLTI); EC 2.1.1.72 (Site-Specific

07159978 PMID: 3522556

Mutant **of** Salmonella **typhimurium LT2 deficient in DNA** adenine **methylation.**

Ritchie L J; Hall R M; Podger D M

Journal of bacteriology (UNITED STATES) Jul 1986, 167 (1) p420-2, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

A **mutant** of **Salmonella** typhimurium LT2 deficient in methylation of the **adenine** residues in the sequence 5'- **GATC** -3' was isolated. The **mutation** (dam-1) was linked to the cysG locus, and the properties of the **mutant** were similar to those of Escherichia coli dam **mutants** . Reversion of the hisC3076 frameshift marker by 9-aminoacridine was substantially enhanced by the dam-1 **mutation** , implying a direct role for **adenine** methylation in the prevention of frameshift **mutation** induction.

Descriptors: ***Adenine** --metabolism--ME; *DNA, Bacterial--metabolism--ME; * **Mutation** ; * **Salmonella typhimurium** -- **genetics** --GE; Aminacrine --pharmacology--PD; **Genes** , Bacterial; Methylation; **Methyltransferases** -- **genetics** --GE; **Methyltransferases** --metabolism--ME; **Salmonella** typhimurium--metabolism--ME; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)

CAS Registry No.: 0 (DNA, Bacterial); 73-24-5 (Adenine); 90-45-9 (Aminacrine)

Enzyme No.: EC 2.1.1. (**Methyltransferases**); EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19860811

Record Date Completed: 19860811

07870810 PMID: 2842672

A mutation **in the DNA** adenine methylase gene **(dam) of** Salmonella **typhimurium decreases susceptibility to 9-aminoacridine-induced frameshift** mutagenesis .

Ritchie L; Podger D M; Hall R M
CSIRO Division of Molecular Biology, North Ryde, NSW, Australia.

Mutation research (NETHERLANDS) Sep 1988, 194 (2) p131-41, ISSN 0027-5107 Journal Code: 0400763
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

A **mutant** of **Salmonella** typhimurium with a reduced response to **mutation** induction by 9-aminoacridine (9AA) has been isolated. The **mutation** (dam-2) is located in the DNA **adenine methylase gene** . The dam-2 **mutant** strain exhibits a level of sensitivity to 2-aminopurine (2AP) intermediate between that of the dam+ and the DNA **adenine** methylation-deficit dam-1 strain, and 2AP sensitivity was reversed by introduction of a mutH **mutation** or of the plasmid pMQ148 (which carries a functional Escherichia coli dam+ **gene**). However, the dam-2 strain is not grossly defective in DNA **adenine methylase** activity. Whole cell DNA appears full methylated at - **GATC** - sites. The levels of 9AA required to induce equivalent levels of frameshift **mutagenesis** in the dam-2 strain were approximately 2-fold higher than for the dam+ strain. Introduction of pMQ148 dam+ reduced the level of 9AA required for induction of frameshift **mutations** 4-fold in the dam-2 strain and 2-fold in the dam+ strain. The dam-2 **mutation** had no effect on the levels of ICR191 required for induction of frameshift **mutations** , but introduction of pMQ148 reduced the ICR191-induced **mutagenesis** 2-fold. The dam+/pMQ148, dam-2/pMQ148 and dam-1/pMQ148 strains showed identical dose-response curves for both 9AA and ICR191. These results are consistent with a slightly reduced (dam-2) or increased (pMQ148) rate of methylation at the replication fork. The 2AP sensitivity of the dam-2 strain cannot be simply explained. Furthermore, addition of methionine to the assay medium reverses the 2AP sensitivity of the dam-2 strain, but has no effect on 9AA **mutagenesis** .

Descriptors: *Aminacrine--pharmacology--PD; *Aminoacridines--pharmacology --PD; * **Genes** , Bacterial; * **Genes** , Structural; * **Methyltransferases** -- **genetics** --GE; * **Mutation** ; * **Salmonella typhimurium** -- **genetics** --GE; DNA Transposable Elements; Genotype; Microbial Sensitivity Tests; **Salmonella** typhimurium--drug effects--DE; **Salmonella** typhimurium--enzymology--EN; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific); Species Specificity

CAS Registry No.: 0 (Aminoacridines); 0 (DNA Transposable Elements);

08462254 PMID: 2155857

DNA methylation in Neisseria **gonorrhoeae and other** Neisseriae .

Ritchot N; Roy P H

Departement de Biochimie, Faculte des Sciences et de Genie, Universite Laval, Sainte-Foy, Canada.

Gene (NETHERLANDS) Jan 31 1990, 86 (1) p103-6, ISSN 0378-1119

Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

It has been reported in the literature that **Neisseria** gonorrhoeae DNA is **modified** by the methyltransferases (MTases) M.NgoI, M.NgoII, and M.NgoIII, as well as three other cytosine MTases and one **adenine** MTase, even if the corresponding restriction endonucleases are not present. We envisioned the possibility of cloning one of the N. gonorrhoeae MTase-encoding **genes** for use as a species-specific DNA probe. We therefore undertook a survey of methylation patterns of several clinical isolates of N. gonorrhoeae and N. meningitidis as well as ATCC strains of other **Neisseriae** . We found, from digestion patterns with isoschizomers, one N. gonorrhoeae strain that lacked M.NgoII and two that lacked M.NgoIII. All N. meningitidis strains (save one) were resistant to digestion with NlaIV thus possessing an MTase like NgoV, and one was resistant to SstII, thus having an NgoIII-like MTase. None were resistant to isoschizomers of NgoI, NgoIII and NgoIV. Some other **Neisseriae** had an MTase with NlaIV (NgoV) specificity, but none had NgoI, II, III or IV specificity, except for the Branhamella-like N. caviae-ovis group and N. lactamica where these specificities were present in at least one strain of this group. Therefore, among the **Neisseriae** other than N. caviae only M.NgoI is N. gonorrhoeae-specific.

Descriptors: *DNA **Modification** **Methylases** --metabolism--ME; * **Neisseria** -- **genetics** --GE; * **Neisseria** gonorrhoeae-- **genetics** --GE; DNA Restriction Enzymes--metabolism--ME; DNA, Bacterial--metabolism--ME; Methylation; **Neisseria** --enzymology--EN; **Neisseria** gonorrhoeae --enzymology--EN

CAS Registry No.: 0 (DNA, Bacterial)

Enzyme No.: EC 2.1.1.- (DNA **Modification** **Methylases**); EC 3.1.21 (DNA Restriction Enzymes)

Record Date Created: 19900426

Record Date Completed: 19900426

08777913 PMID: 2277628

Cloning and characterization of two tandemly arranged DNA methyltransferase genes **of** Neisseria **lactamica: an** adenine **-specific M.NlaIII and a cytosine-type** methylase .

Labbe D; Holtke H J; Lau P C
Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec.

Molecular & general genetics - MGG (GERMANY) Oct 1990, 224 (1) p101-10, ISSN 0026-8925 Journal Code: 0125036
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The **gene** encoding the **Neisseria** lactamica III DNA **methyltransferase** (M.NlaIII) which recognizes the sequence CATG has been cloned and expressed in Escherichia coli. DNA sequencing of a 3.125 kb EcoRI-PstI fragment localizes the M. NlaIII **gene** to a 334 codon open reading frame (ORF) and identifies, 468 bp downstream, a second ORF of 313 amino acids, which is referred to as M.NlaX. Both proteins are detectable in the E. coli coupled in vitro transcription-translation system; they are apparently expressed from separate N. lactamica promoters. The N-terminal half of the previously characterized M.FokI, which methylates **adenine** in one of the DNA strands with its asymmetric recognition sequence (GGATG), is found to have 41% sequence identity and a further 11.7% sequence similarity with M.NlaIII. Among the conserved amino acids is the wellknown DPPY sequence motif. With one exception, analysis of the nucleotides coding for the DP dipeptide in all known DPPY sequences shows the presence of an inherent DNA **adenine** methylation (dam) recognition site of **GATC** . A low level of expression of M.NlaX in E. coli prevents the elucidation of its sequence recognition specificity. Sequence analysis of M.NlaX shows that it is closely related to the group of monospecific 5-methylcytosine DNA **methyltransferases** (M.EcoRII, Dcm, M.HpaII and M.HhaI) which all have a **modified** cytosine at the second position of the recognition sequences. Both M.EcoRII and Dcm amino acid sequences are about 50% identical with M.NlaX; a considerable degree of sequence identity is found in the so-called variable region which is believed to be responsible for sequence recognition specificity. M.NlaX is probably the counterpart to the E. coli Dcm in N. lactamica.

Tags: Support, Non-U.S. Gov't
Descriptors: ***Neisseria** -- **genetics** --GE; *Site-Specific DNA **Methyltransferase** (Cytosine-Specific)-- **genetics** --GE; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)-- **genetics** --GE; Amino Acid Sequence; Base Sequence; Cloning, Molecular; **Genes** , Bacterial; Molecular Sequence Data; **Neisseria** --enzymology--EN; Open Reading Frames; Restriction Mapping; Sequence Homology, Nucleic Acid; Site-Specific DNA **Methyltransferase** (Cytosine-Specific)--metabolism--ME; Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)--metabolism--ME

08367707 PMID: 2688642

A mutation **in the dam** gene **of** Vibrio **cholerae: 2-aminopurine sensitivity with intact** GATC methylase **activity**.

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Biophysics Division, Indian Institute of Chemical Biology, Calcutta.

Biochemical and biophysical research communications (UNITED STATES) Dec 15 1989, 165 (2) p561-7, ISSN 0006-291X Journal Code: 0372516

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Vibrio cholerae **mutants** sensitive to 2-aminopurine (2AP) but with DNA **adenine** **methylase** activity similar to parental cells have been isolated. The **mutant** strains were sensitive to ultraviolet light (UV), methyl methane sulphonate (MMS) and 9-aminoacridine. The spontaneous **mutation** frequency of the **mutants** were not significantly affected. Attempts to isolate dam V. cholerae cells by screening 2AP sensitive cells have not been successful. All the **mutant** phenotypes could be suppressed by introducing the plasmid pRB103 carrying the dam **gene** of Escherichia coli into the **mutant** cells.

Tags: Support, Non-U.S. Gov't

Descriptors:. *2-Aminopurine--pharmacology--PD; * **Adenine** --analogs and derivatives--AA; * **Genes** , Structural, Bacterial; * **Mutation** ; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific)-- **genetics** --GE ; * **Vibrio** cholerae-- **genetics** --GE; Aminacrine--pharmacology--PD; Bacteriophages-- **genetics** --GE; Base Sequence; Conjugation, **Genetic** ; Escherichia coli-- **genetics** --GE; Kinetics; Methyl Methanesulfonate --pharmacology--PD; Plasmids; Substrate Specificity; Ultraviolet Rays; **Vibrio** cholerae--drug effects--DE; **Vibrio** cholerae--radiation effects --RE

CAS Registry No.: 0 (Plasmids); 452-06-2 (2-Aminopurine); 66-27-3 (Methyl Methanesulfonate); 73-24-5 (Adenine); 90-45-9 (Aminacrine)

Enzyme No.: EC 2.1.1.72 (Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific))

Record Date Created: 19900124

Record Date Completed: 19900124

United States Patent [19]

Anderson et al.

[11] Patent Number: 4,798,791

[45] Date of Patent: Jan. 17, 1989

[54] VECTOR FOR HIGH LEVEL GENE EXPRESSION

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[21] Appl. No.: 671,967

[22] Filed: Nov. 16, 1984

[51] Int. Cl.[4] C12P 21/00; C12P 19/34; C12N 5/00; C12N 7/00

[52] U.S. Cl. 435/68; 435/70; 435/91; 435/172.3; 435/317; 435/320; 935/6; 935/22; 935/29; 935/48; 935/73; 536/27

[58] Field of Search 435/68, 71, 91, 253, 435/317, 172.3; 536/27; 935/6, 29, 22, 41, 48, 56, 72, 40, 60, 73

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Assistant Examiner—S. Seidman
Attorney, Agent, or Firm—Saidman, Sterne, Kessler & Goldstein

[57] ABSTRACT

Methods and vectors for high level expression of genes in bacteria are disclosed. A terminal mRNA sequence from a gene coding for a stable bacterial protein mRNA is ligated to a gene coding for the desired protein adjacent the translation termination codon of the gene. The gene for the desired protein and the terminal mRNA sequence are situated in an expression vector in which the gene is operably linked to a transcription promoter.

23 Claims, 2 Drawing Sheets

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FIG. 1.

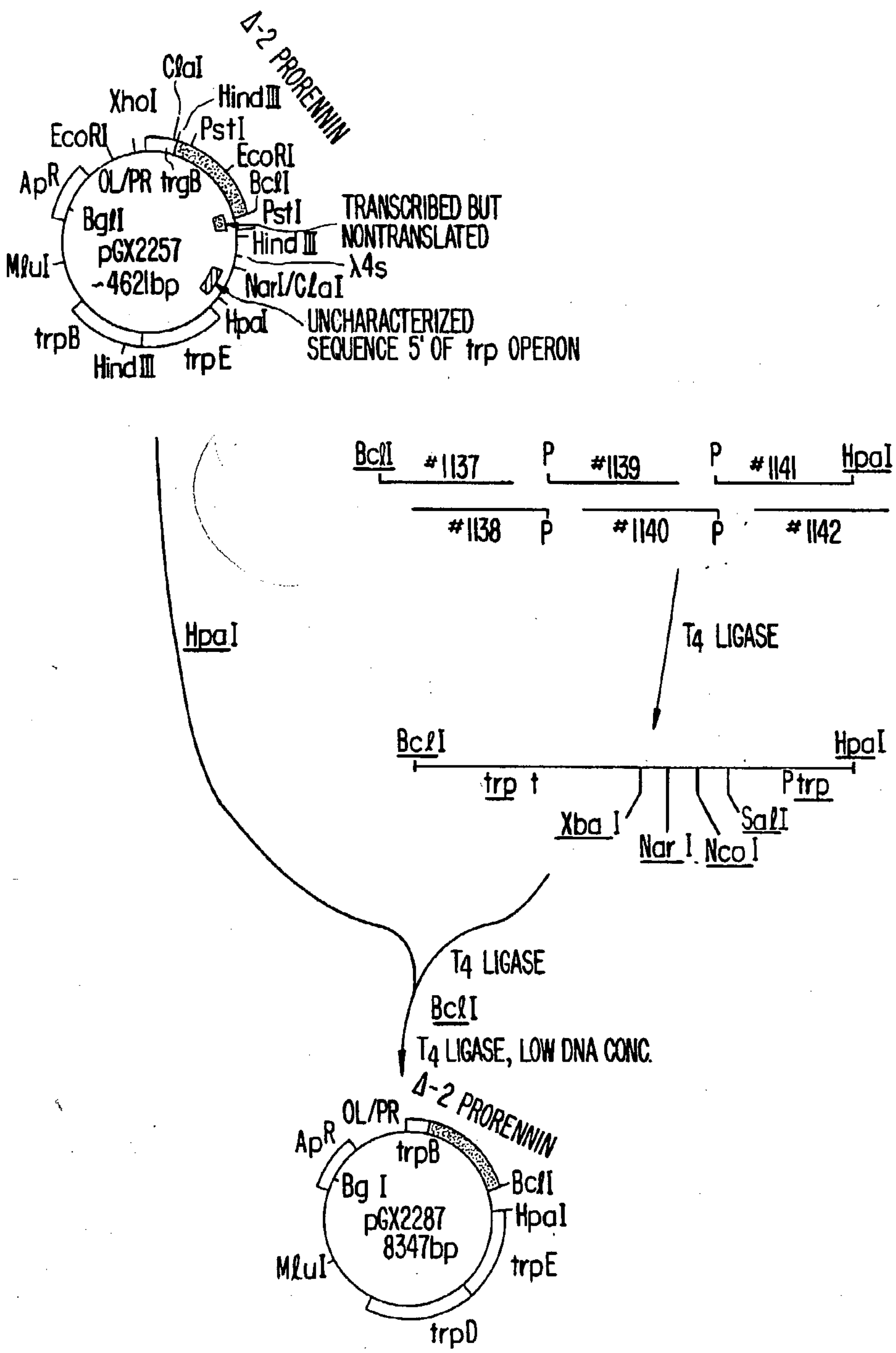
Δ-2 PRORENNIN
ClaI
XhoI
HindIII
PstI
EcoRI
EcoRI
BclI
PstI
ApR
OL/PR trgB
BglI
pGX2257
~4621bp
MluI
TRANSCRIBED BUT
NONTRANSLATED
HindIII
λ4s
NarI/ClaI
HpaI
UNCHARACTERIZED
SEQUENCE 5' OF trp OPERON
trpB
HindIII
trpE
BclI
#1137
P
#1139
P
#1141
HpaI
#1138
P
#1140
P
#1142
HpaI
T4 LIGASE
BclI
HpaI
trp t
Ptrp
XbaI
NarI
NcoI
SalI
T4 LIGASE
BclI
T4 LIGASE, LOW DNA CONC.
Δ-2 PRORENNIN
OL/PR
ApR
trpB
Bg I
pGX2287
8347bp
BclI
HpaI
MluI
trpE
trpD

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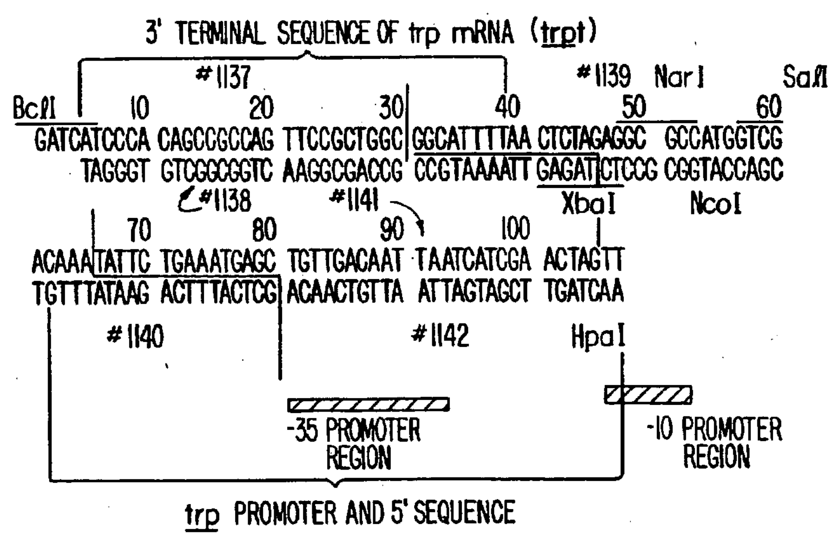
FIG. 2.

DNA SEQUENCE OF ASSEMBLED OLIGONUCLEOTIDES FOR CHYMOSIN 3' END



VECTOR FOR HIGH LEVEL GENE EXPRESSION

BACKGROUND OF THE INVENTION

The use of recombinant DNA technology has made possible the large scale production in fermentation facilities of proteins which would otherwise have to be isolated from natural sources. In the application of this technology, a microorganism such as a bacterium is transformed with a replicable expression vector containing a gene which codes for a protein not normally produced by the bacterial host, i.e., a heterologous protein. The gene for the heterologous protein is operably linked to a regulatory sequence of DNA, including a transcription promoter, which is capable of directing expression of the gene in the bacterial host. Due to the universality of the genetic code, the bacterial host is capable of transcribing the heterologous gene into messenger RNA (mRNA) and then translating the mRNA into protein having the amino acid sequence of the heterologous protein.

Over the last several years, a great deal of research in the recombinant DNA field has focused on methods for improving the efficiency with which transformed microorganisms can be made to express heterologous genes in order to improve yields of desired products. Efforts at improving levels of heterologous gene expression have been primarily directed at manipulation of "upstream" DNA components—that is, DNA sequences which precede the heterologous gene in the expression vector and affect the frequency and efficiency of transcription—and at controlling post-translational events which affect product stability or recovery. The latter approach is exemplified by efforts to develop host-vector combinations which effect secretion of the translated heterologous protein through the cell wall of the host cell and into the surrounding medium where it is less subject to degradative attack by proteases produced in the host cell.

While considerable improvements have been made in obtainable levels of gene expression and product recovery, the art continues to seek methods by which heterologous genes can be expressed and the expression products recovered in increased amounts.

SUMMARY OF THE INVENTION

This invention provides methods for expressing genes coding for desired proteins in bacteria at elevated levels of expression. In accordance with the method of the invention, the 3' terminal sequence found in the mRNA from a gene coding for a protein native to the bacterial host is ligated, in the form of its complementary DNA, to a structural gene for the desired protein adjacent the translation termination signal of the gene. The gene for the desired protein having the terminal mRNA nucleotide sequence ligated thereto is situated in a vector that is replicable in the bacterial host such that the gene is operably linked to a transcription promoter. The bacterium is then transformed with this vector. The transformant is grown up and the gene expressed using conventional techniques. A preferred terminal mRNA nucleotide sequence for use in the method of the invention is the trpt sequence of the *E. coli* tryptophan operon.

There is also provided a replicable expression vector for expressing a desired protein in a bacterium. The replicable expression vector comprises a structural gene for any desired protein operably linked to a transcription promoter; and a terminal mRNA nucleotide sequence from a gene coding for a protein native to a bacterium, said terminal sequence being adjacent the translation termination codon of the desired gene.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of the construction of plasmid pGX2287, which contains the trpt sequence just 3' to the translation termination sequence of a gene coding for Δ-2 prochymosin.

FIG. 2 illustrates the DNA sequence of assembled synthetic oligodeoxynucleotides, including the trypt sequence, which were inserted adjacent the translation termination codon of the Δ-2 prochymosin gene.

DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

While not wishing to be bound by any particular theory of the mechanism by which increased expression levels are effected in accordance with the invention, it is believed that the insertion of a terminal mRNA sequence from a bacterial gene at the 3' end of the desired gene, i.e., just downstream from the translation termination signal, results in production of mRNA of increased stability.

In vitro evidence suggests that the *E. coli* trpt sequence may not be a strong terminator of transcription (Holmes, et al., *Cell*, 32:1029–1032 [1983]) and that transcription termination of the trp operon in *E. coli* may occur in vivo downstream from the trpt sequence (Guarente, et al., *J. Mol. Biol.*, 133:189–197 [1979]). One interpretation of these data is that transcription termination takes place 3' of trpt followed by rapid degradation of mRNA up to the trpt sequence. That is, the trypt sequence may act as a degradation stop signal, thereby stabilizing the mRNA. By placing a terminal mRNA sequence such as trpt at the end of a heterologous gene, degradation of mRNA into the structural gene is inhibited. Advantageously, insertion of the trpt sequence is accompanied by removal of normally transcribed but untranslated DNA at the 3' end of the heterologous gene. Removal of untranslated sequence at the 3' end of the gene may reduce the probability of nuclease sensitive sites being present in the mRNA and avoids expending energy on the synthesis of unnecessary mRNA.

In accordance with the practice of the invention, a terminal mRNA sequence of a gene coding for a stable bacterial protein mRNA is ligated to the 3' end of the gene for the desired protein. As used herein, the phrase "terminal mRNA sequence" refers to the untranslated portion of a gene for a bacterial protein that codes for the 3' end of a stable mRNA for the bacterial protein. The terminal mRNA sequence may or may not be the point at which transcription actually terminates during formation of the bacterial protein mRNA. If it is not, however, it corresponds to the 3' terminus of the stable form of the mRNA.

Preferably, the terminal mRNA sequence is derived from a gene for a protein which is produced in relative abundance by the bacterial host. Suitable terminal mRNA sequences for use in the invention include the trypt sequence of the *E. coli* tryptophan operon, and terminal RNA sequences from mRNA that codes for abundant *E. coli* proteins such as the omp, rpo, rps, rpm, rpl genes or other genes terminal in operons that code for abundant proteins or proteins that become abundant after induction. Unlike trypt, in some cases the actual terminal stable mRNA sequence has not yet

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been determined. Thus, one could either determine the terminal sequence of isolated mRNA using known techniques or utilize the entire sequence between the termination codon and apparent transcription termination sites predicted from DNA sequence. It is preferable, however, to utilize a characterized sequence that has been determined at the end of a stable mRNA since it may not always be possible to accurately predict transcription termination sites from DNA sequence (especially rho dependent termination sites). Preferably, the terminal mRNA sequence employed is the trpt sequence of the *E. coli* tryptophan operon. This is a 34-nucleotide DNA sequence which follows the stop codon (TAA) at the end of the trpA gene.

Referring to FIG. 2, the trpt sequence is that portion of the synthetic oligodeoxynucleotide beginning at nucleotide no. 5, ATCC . . . , and ending at nucleotide no. 39, . . . TTTT. The terminal mRNA sequence which is inserted at the 3′ end of the heterologous gene is preferably obtained by techniques of oligonucleotide synthesis. We produced the oligonucleotides shown in FIG. 2 using standard phosphoramidite methods (S. L. Beaucage and M. H. Caruthers, *Tetrahedron Lett.*, 22:1859 [1981]); M. D. Matteucci and M./ H. Caruthers, *Tetrahedron Lett.*, 21:719 [1980]); M. D. Matteucci and M. H. Caruthers, *J. Am. Chem. Soc.*, 103:3185 [1981]) and a commercially available oligonucleotide synthesis machine from Applied Biosystems, Inc.

While the invention is exemplified below with respect to expression of prochymosin, it will be readily understood that the methods of the invention are equally applicable to the expression of genes coding for virtually any heterologous protein or even a non-heterologous protein (different from the protein corresponding to the terminal mRNA sequence), where expression amplification of the non-heterologous gene is desired. Many such genes have already been cloned and inserted into expression vectors in which they are operably linked to transcription promoters and other regulatory sequences which direct and control gene expression in bacterial hosts. Any known expression vectors, including plasmids and phages, which are capable of replication in bacterial hosts, can be employed in the practice of the invention. Likewise, any known promoters which are capable of directing transcription of heterologous genes in bacterial hosts can be employed. These include, for example, the trp promoter, trp/lac hybrid promoter, λ O_L/P_R promoter (derived from the leftward operator and rightward promoter of phage λ) and other known promoters.

The expression vector of the invention can be produced by modification of an existing expression vector containing a gene for the desired protein operably linked to a transcription promoter. Referring to FIG. 1, we produced an expression vector of the invention for high level expression of prochymosin (pGX2287) starting with plasmid pGX2257. Plasmid pGX2257 contains a structural gene for Δ-2 prochymosin (prochymosin less its first two amino acids), fused at its 5′ end to a trpB gene, under the control of the λ O_L/P_R hybrid promoter. The Δ-2 prochymosin gene in pGX2257 contains a 228-base pair transcribed but untranslated region following the translation termination codon. The untranslated region was removed by endonuclease cleavage, first with HpaI and then with BclI. A synthetic oligonucleotide (FIG. 2) containing the trpt sequence on a BclI-HpaI fragment, was inserted immediately downstream from the translation termination codon. The insert was constructed to contain a number of convenient restriction sites 3′ to the trpt sequence so that the Δ-2 prochymosin gene and trpt sequence could easily be transferred to other vectors.

It is preferred to insert the terminal transcription sequence of the bacterial protein as close as possible to the 3′ end of the translation termination codon of the gene for the desired protein. It may be difficult or impossible to have it contiguous with the translation termination signal without the use of synthetic DNA, site directed mutagenesis, or exonuclease treatment depending on the location of convenient endonuclease sites. The requirement that the terminal mRNA sequence be "adjacent the translation termination signal" of the gene for the desired protein is satisfied if there are no more than about 100 nucleotides, preferably no more than about 3 nucleotides, separating the last nucleotide of the translation termination codon of the gene for the desired protein and the first nucleotide of the terminal mRNA sequence from the stable bacterial protein mRNA.

The expression vector of the invention containing the gene coding for the desired protein operably linked to a transcription promoter and the terminal mRNA sequence adjacent to the translation termination signal is used to transform a bacterial host such as an *E. coli* in a conventional manner. The transformed bacteria are then grown up in a fermentor and subjected to conditions under which the gene for the desired protein is expressed. We have found that *E. coli* strain GX1731 transformed with plasmid pGX2287 produced considerably higher titers of prochymosin than the same host strain transformed with the parental plasmid, pGX2257, under similar fermentation conditions.

The following examples are intended to further illustrate the practice of the invention and are not intended to limit the scope of the invention in any way.

EXAMPLE 1

Construction of Plasmid pGX2287

Referring to FIG. 2, each of the single-strand oligonucleotides identified as #1137, 1138, 1139, 1140, 1141 and 1142 were synthesized using standard phosphoramidite methods (S. L. Beaucage and M. H. Caruthers, *Tetrahedron Lett.*, 22:1859 (1981); M. D. Matteucci and M. H. Caruthers, *Tetrahedron Lett.*, 21:719 (1980); M.D. Matteucci and M. H. Caruthers, *J. Am. Chem. Soc.*, 103:3185 (1981)) and a commercially available oligonucleotide synthesis machine (Applied Biosystems). The oligonucleotides were purified using HPLC and adjusted to a concentration of 1.0 A_{260} unit per ml of H_2O. One microgram (20 μl) of oligonucleotides #1138, 1139, 1140 and 1141 were phosphorylated in a volume of 100 μl using T_4 polynucleotide Kinase (11 units, Boehringer Mannheim) using 1 mM ATP and the buffer conditions recommended by the manufacturer for 12 hours and 40 minutes. The four Kinase reactions were boiled for one minute to inactivate Kinase, then pooled. One microgram (20 μl) of oligonucleotides #1137 and 1142 were added and the reaction volume was adjusted to 500 μl with the buffer recommended for T_4 DNA ligase. T_4 DNA ligase (2×10^6 units, New England Biolabs) was added and the mixture was incubated at 15° C. for 12 hours. Analysis of an aliquot of the ligation by gel electrophoresis demonstrated the presence of a DNA fragment of approximately 100 bp as expected. The fragment contained the

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trpt sequence followed by restriction sites for XbaI, NarI, NcoI and SalI, respectively. The 3′ end of the fragment contained components of the trp promoter. This promoter is present in order to promote transcription of the trpED stabilization genes in pGX2257 (see FIG. 1). The stabilization genes stabilize the plasmid in host strain GX1731 by plasmid complementation of host cell mutation.

Plasmid pGX2257 DNA was prepared from *E. coli* strain GX3003 (pGX2257) that contains a DNA adenine methylase (dam) mutation. *E. coli* strain GX1731 (pGX2257) has been deposited at the U.S. Department of Agriculture, Northern Regional Research Laboratory, Peoria, Ill., with accession No. NRRL B-15771. Any *E. coli* host with a dam mutation (A. Bale, M. D'Alarcao and M. G. Marinus, *Mutation Research*, 59:157165 (1979)) that is a λ phage lysogen can be utilized in place of GX3003 for this method. The pGX2257 DNA (15 μg) prepared in a dam host was digested with 8 units of HpaI endonuclease in 100 μl of the buffer recommended by the manufacturer at 37° C. for two hours. The DNA solution was then extracted with a 1:1 mixture of water saturated phenol and cloroform (adjusted to pH 8.0 with tris-[hydroxymethyl]-aminomethane base). The aqueous phase was adjusted to a 0.2 M sodium acetate pH 5.5 and 2.5 volume of 95% ethanol was added to precipitate the DNA. The ethanol solution was frozen using dry ice then centrifuged at 15,000 X G to precipitate the DNA. The DNA was dissolved in 30 μl H_2O to give a DNA concentration of 0.5 μg/μl.

Referring to FIG. 1, the ligated synthetic DNA described above was ligated to HpaI-cut pGX2257 DNA by addition of 5 μl(2.5 μg) of HpaI-cut pGX2257 to 60 μl (~0.72 μg) of the oligonucleotide ligation in a total reaction volume of 75 μl with 8×10^5 units of T_4 DNA ligase at 15° C. with 0.5 mM ATP for 12 hours. The ligation was phenol-chloroform extracted and ethanol precipitated as described above, then dissolved in 50 μl H_2O. The ligated DNA was then digested with 12 units of BclI endonuclease in 70 μl of buffer recommended by the manufacturer at 37° C. for 2 hours. The BclI digestion reaction was phenol-chloroform extracted and the DNA was precipitated as described above. The DNA pellet was dissolved in 80 μl of H_2O. The final ligation at low DNA concentration to circularize the DNA included 15 μl of the BclI-cut DNA in a 150 μl ligation reaction with 8×10^5 units of T_4 DNA ligase, 1 mM ATP in the recommended buffer at 15° C. for 12 hours. Aliquots of the final ligation reaction were used to transform *E. coli* GX1731 (F−, [λcI857-ΔBAMΔHI]Δ[chlA-pgl]nadA::Tn10 ΔtrpED102 tna2) using the standard calcium shock procedure. Plasmids in transformant colonies were screened using agarose gel electrophoresis looking for plasmids with decreased size relative to pGX2257 and the presence of XbaI, NarI, NcoI and SalI endonuclease sites that are located in the synthetic DNA. Cells containing plasmids with the correct endonuclease digestion pattern were tested for the ability to grow independent of tryptophan to demonstrate plasmid complementation of the host trp deletion. One plasmid with the proper characteristics was labeled pGX2287. Strain GX1731, transformed with pGX2287, was deposited at the U.S. Department of Agriculture, Northern Regional Research Laboratory, Peoria, Illinois, with accession No. NRRL B-15788.

EXAMPLE 2

Expression of trpB/Δ-2 Prochymosin

A series of fermentation runs was conducted, using host strain GX1731, transformed with either pGX2287 or its parental plasmid pGX2257. In each instance, a liquid nitrogen stored ampule of the transformant strain was thawed and 0.4 ml of the contents were inoculated into each of two 250 ml baffled flasks containing 50 ml of LB broth supplemented with 100 μg/ml ampicillin. The two flasks were incubated at 30° C. and 250 rpm in an incubator shaker for 6.5 to 9 hours.

Fermentation was carried out using 8 liters of the following initial medium:

$(NH_4)_2SO_4$	30 g
KH_2PO_4	15 g
K_2HPO_4	5 g
Biotin (0.5 mg/ml in 95% Ethanol)	12 ml
Tap water to 8 liters, autoclave	

$CaCl_2.2H_2O$	10 ml of 10% (w/v) sterile solution
glucose	360 ml of 50% (w/v) sterile solution
niacon	18 ml of 0.5% (w/v) sterile solution
Trace solution 1	90 ml
Trace solution 2	18 ml
Trace solution 3	1.8 ml

The following fermentation conditions were maintained:

pH 7.0 (controlled by NH_4OH, 5N and H_3PO_4, 1N)	
Sparge rate	1 vvm
Temperature	32° C.
Agitation rate	800 rpm

The initial medium was inoculated with the contents of the baffled flask to a final volume of 9.0 liters.

In order to increase cell density prior to induction of expression, a system of broth supplementation with nutrients was undertaken. The feed solution was prepared as follows:

1000 g glucose with deionized water for final volume of 1700 mls was autoclaved. After autoclaving, there was added:

Trace solution 1	500 ml
Trace solution 2	100 ml
Trace solution 3	10 ml
$CaCl_2.2H_2O$	50 ml
Trace Solution #1	
H_2O	900 ml
conc HCl	13.3 ml
$FeCl_3.6H_2O$	5.4 g
$ZnSO_4.7H_2O$	1.44 g
$MnCl_2.4H_2O$	1.0 g
$CuSO_4.5H_2O$	0.25 g
$CoCl_2.6H_2O$	0.24 g
H_3BO_3	0.062 g
Brought to 1000 ml and sterile filtered	
Trace Solution #2	
H_2O	900 ml
HCl	44.8 ml
$MgSO_4.7H_2O$	61.6 g
Brought to 1000 ml and sterile filtered	

-continued

Trace Solution #3	
H_2O	1000 ml
$Na_2MoO_4.2H_2O$	24.1 g
Sterile filtered	

The feed solution was initially added to the broth in a volume of 180 ml and thereafter as needed to maintain the glucose level at 10 g/liter. Feed supplementation was continued until the A_{600} reached 20, at which time the cells were induced to express trpB/Δ-2 prochymosin. Induction was effected by raising the temperature to 42° C. to deactivate the temperature sensitive cI857 repressor protein. After one hour, the temperature was reduced to 39° C. for the remainder of the run (about six hours).

One-ml samples of cells from fermentors were placed in 1.5 ml Eppendorf tubes and centrifuged for 5 minutes. Cell pellets were resuspended in 1 ml of water, recentrifuged, and stored as pellets at −20° C.

Cell pellets were suspended in water at an A_{600} of 10 in 15 ml Corex tubes. Two ml of cells at A_{600} = 10 were lysed by sonication for 6 pulses of 30 seconds each. The suspension was kept on ice during sonication. The A_{600} of uninduced cells was about 0.5 after sonication.

The cell sonicate was centrifuged at 8000 rpm for 15 minutes (SS34 rotor). The supernatant was discarded, and the pellet suspended in 90 μl of 10 mM EDTA by vortexing. 10 μl of 1 N NaOH was added, and the suspension was vortexed until it dissolved. There was added 0.8 ml of water followed by 0.1 ml of 0.1 M glycine, pH 7.5, and the solution was incubated overnight at room temperature. The pH at this point was 10.0. The pH was then adjusted to 2.0 by addition of about 20 μl of 1 N HCl. Incubation was continued for two hours at room temperature. Under these conditions, trpB/Δ-2 prochymosin underwent autocatalytic cleavage to produce mature, active chymosin.

Chymosin activity was evaluated by observing the formation of a parakappa casein precipitate following the catalytic cleavage of kappa casein to give parakappa casein and a small soluble glycoprotein. Chymosin activity was calculated from the time required for the absorbance at 600 nm (light scattering) of kappa casein substrate to reach 1.0. Activity is expressed relative to commercial single-strength rennet, which is used as a standard and defined to have an activity of 1.0 U/ml.

The results from each of four fermentation runs using a strain transformed with plasmid pGX2287 and four fermentation runs using a strain transformed with parental plasmid pGX2257, which lacks the trpt sequence, are given in the table below. It can be seen from the table that the strain transformed with plasmid pGX2287, which contains the trpt sequence immediately downstream from the translation termination signal of the Δ-2 prochymosin gene, produced several-fold higher titers of chymosin activity than the parental strain, pGX2257.

Plasmid	Run	Chymosin Activity (Units/Liter)
pGX2257	1	35
	2	22
	3	18
	4	23
pGX2287	5	83
	6	75
	7	88
	8	113

What is claimed is:

1. A method for expressing a gene encoding a desired protein in an *E. coli* host at elevated levels of expression, which comprises:
   (a) inserting a structural gene encoding a desired protein and the trpt sequence of the *E. coli* tryptophan operon into a vector replicable in an *E. coli* host, such that said sequence is adjacent to and downstream from the translation termination codon of said structural gene;
   (b) transforming said *E. coli* host with said vector; and
   (c) expressing said structural gene in the thus transformed *E. coli* host.

2. The method of claim 1, wherein said desired protein is a heterologous protein.

3. The method of claim 1, wherein said desired protein is a homologous protein other than *E. coli* tryptophan synthase A protein.

4. The method of claim 2, wherein said heterologous protein is prochymosin.

5. The method of claim 1, wherein said trpt sequence comprises the trpt sequence following the stop codon of the trpA gene.

6. The method of claim 5, wherein said trpt sequence is

ATCCCACAGCCGCCAGTTCCGCTGGCG-GCATTTT.

7. The method of claim 1, wherein said vector is the vector pGX2287.

8. The method of claim 1, wherein said *E. coli* host is *E. coli* strain GX1731.

9. The method of claim 1, wherein all or a portion of the transcribed but untranslated region normally present at the 3' end of said structural gene is removed.

10. A replicable expression vector for expressing a desired protein in an *E. coli* host at elevated levels of expression, which comprises the trpt sequence of the *E. coli* tryptophan operon.

11. The vector of claim 10, wherein said trpt sequence comprises a 34 nucleotide sequence which follows the stop codon of said trpA gene.

12. The vector of claim 11, wherein said 34 nucleotide sequence comprises the following sequence:

ATCCCACAGCCGCCAGTTCCGCTGGCG-GCATTTT.

13. The vector of claim 10, further comprising a structural gene encoding a desired protein, wherein said first trpt sequence is adjacent to and downstream from the translation termination codon of said structural gene.

14. The vector of claim 13, wherein said desired protein is a heterologous protein.

15. The vector of claim 13, wherein said desired protein is a homologous protein other than *E. coli* tryptophan synthase A protein.

16. The vector of claim 14, wherein said heterologous protein is prochymosin.

17. The vector of claim 13, wherein said structural gene is devoid of all or a portion of the transcribed but untranslated region normally present at the 3' end of said gene.

18. The vector of claim 10, wherein said vector is a plasmid.

19. The vector of claim 18, wherein said plasmid is pGX2287.

20. An *E. coli* host transformed with the vector of claim 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19.

21. The *E. coli* host of claim 20, wherein said host is *E. coli* strain GX1731.

22. *E. coli* strain GX1731, transformed with vector pGX2287.

23. The plasmid pGX2287.

* * * * *

DOCUMENT-IDENTIFIER: US 4798791 A
TITLE: Vector for high level gene expression

Detailed Description Text (15):
Plasmid pGX2257 DNA was prepared from E. coli strain GX3003 (pGX2257) that contains a DNA adenine methylase (dam) mutation. E. coli strain GX1731 (pGX2257) has been deposited at the U.S. Department of Agriculture, Northern Regional Research Laboratory, Peoria, Ill., with accession No. NRRL B-15771. Any E. coli host with a dam mutation (A. Bale, M. D'Alarcao and M. G. Marinus, Mutation Research, 59:157165 (1979)) that is a .lambda. phage lysogen can be utilized in place of GX3003 for this method. The pGX2257 DNA (15 .mu.g) prepared in a dam host was digested with 8 units of HpaI endonuclease in 100 .mu.l of the buffer recommended by the manufacturer at 37.degree. C. for two hours. The DNA solution was then extracted with a 1:1 mixture of water saturated phenol and cloroform (adjusted to pH 8.0 with tris-[hydroxymethyl]-aminomethane base). The aqueous phase was adjusted to a 0.2 M sodium acetate pH 5.5 and 2.5 volume of 95% ethanol was added to precipitate the DNA. The ethanol solution was frozen using dry ice then centrifuged at 15,000 X G to precipitate the DNA. The DNA was dissolved in 30 .mu.l H.sub.2 O to give a DNA concentration of 0.5 .mu.g/.mu. l.

12694301 PMID: 7616563

Pyrimidine regulation of the Escherichia coli and Salmonella **typhimurium carAB operons: CarP and integration host factor (IHF) modulate the methylation status of a** GATC **site present in the control region.**

Charlier D; Gigot D; Huysveld N; Roovers M; Pierard A; Glansdorff N
Research Institute of the CERIA-COOVI, Brussels, Belgium.

Journal of molecular biology (ENGLAND) Jul 21 1995, 250 (4) p383-91, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

By measuring the protection against Dam **methylase modification** of a **GATC** sequence located 106 bp upstream of the startpoint of promoter P1 in the control region of the carAB operon (encoding carbamoylphosphate synthetase) we have obtained evidence for a direct correlation between the degree of in vivo occupancy of a specific regulatory target site and the repressibility of the P1 promoter by pyrimidine residues. A high uridine nucleotide pool as well as binding of the carP (alias xerB/pepA) **gene** product and of the integration host factor (IHF) to the carAB control region are prerequisites to observe this in vivo protection. Purified CarP binds in vitro to the carAB control region and protects against DNase I two approximately 25 bp long stretches, one of which is located just downstream of the **GATC** sequence. **Mutations** in this site strongly impair the pyrimidine regulation of the P1 promoter and the interference with Dam **methylase modification** . These processes are also strongly impaired in the absence of integration host factor and in **mutants** affected in the IHF site located some 200 bp upstream of this Dam **methylase modification** site. IHF therefore exerts at least part of its antagonistic effects on P1, i.e. increased expression in minimal medium but increased repression in the presence of pyrimidine residues, indirectly by influencing the formation or the stability of a particular protein-DNA complex. Furthermore, we demonstrate that the distance separating the IHF and Dam **methylase** target sites is crucial for the in vivo protection and for pyrimidine-mediated regulation of the promoter expression. **Mutations altering** this distance result in severe reductions of the degree of in vivo protection and, concomitantly, of the repressibility by pyrimidine residues of promoter P1 activity in a way indicative of the formation of a complex nucleoprotein structure. Since neither IHF nor CarP require pyrimidine residues to bind to the carAB control region, at least not in vitro, it is tempting to suggest that IHF and CarP-induced bending and looping provide changes in DNA topology that are required for assembling a specific pyrimidine-dependent nucleoprotein complex that modulates P1 activity.

Tags: Support, Non-U.S. Gov't

Descriptors: *Aminopeptidases--metabolism--ME; *Bacterial Proteins --metabolism--ME; *Carbamoyl-Phosphate Synthase (Glutamine-Hydrolyzing)-- **genetics** --GE; *Promoter Regions (**Genetics**); *Pyrimidines--metabolism --ME; *Site-Specific DNA- **Methyltransferase** (**Adenine** -Specific) --metabolism--ME; **Adenine** --metabolism--ME; Aminopeptidases-- **genetics** --GE; Animals; Bacterial Proteins-- **genetics** --GE; B

DNA-Methyltransferase (Adenine-Specific)); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.21.- (endodeoxyribonuclease StyLTI); EC 3.1.21.5 (Deoxyribonucleases, Type III Site-Specific)
Gene Symbol: mod; res
Record Date Created: 19910311
Record Date Completed: 19910311

12/9/6
DIALOG(R)File 155:MEDLINE(R)
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13632145 PMID: 9321671
The Neisseria gonorrhoeae S.NgoVIII restriction/modification system: a type IIs system homologous to the Haemophilus parahaemolyticus HphI restriction/modification system.
Gunn J S; Stein D C
University of Maryland, Department of Microbiology, College Park, MD 20142, USA.
Nucleic acids research (ENGLAND) Oct 15 1997, 25 (20) p4147-52, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: AI24452; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Strains of Neisseria gonorrhoeae possess numerous restriction-**modification** (R-M) systems. One of these systems, which has been found in all strains tested, encodes the S. NgoVIII specificity (5'TCACC 3') R-M system. We cloned two adjacent methyltransferase **genes** (dcmH and damH), each encoding proteins whose actions protect DNA from digestion by R.HphI or R.Ngo BI (5'TCACC 3'). The damH **gene** product is a N 6-methyladenine methyltransferase that recognizes this sequence. We constructed a plasmid containing multiple copies of the S.NgoVIII sequence, grew it in the presence of damH and used the HPLC to demonstrate the presence of N 6-methyladenine in the DNA. A second plasmid, containing overlapping damH and Escherichia coli dam recognition sequences in combination with various restriction digests, was used to identify which adenine in the recognition sequence was **modified** by damH. The predicted dcmH **gene** product is homologous to 5-methylcytosine methyltransferases. The products of both the dcmH and damH **genes** , as well as an open reading frame downstream of the damH **gene** are highly similar to the **Haemophilus** parahaemolyticus hphIMC , hphIMA and hphIR **gene** products, encoding the Hph I Type IIs R-M system. The S.NgoVIII R-M **genes** are flanked by a 97 bp direct repeat that may be involved in the mobility of this R-M system.
Tags: Comparative Study; Support, U.S. Gov't, P.H.S.
Descriptors: *DNA Restriction-Modification Enzymes--genetics--GE; *Haemophilus--genetics--GE; *Neisseria gonorrhoeae--genetics--GE; *Site-Specific DNA Methyltransferase (Cytosine-Specific)--genetics--GE; Amino Acid Sequence; Cloning, Molecular; DNA, Bacterial--chemistry--CH; DNA, Bacterial--genetics--GE; Haemophilus--enzymology--EN; Molecular Sequence Data; Neisseria gonorrhoeae--enzymology--EN; Open Reading Frames; Restriction Mapping; Sequence Analysis, DNA; Sequence Homology; Site-Specific DNA Methyltransferase (Cytosine-Specific)--chemistry--CH
Molecular Sequence Databank No.: GENBANK/AF001598
CAS Registry No.: 0 (DNA Restriction-Modification Enzymes); 0 (DNA, Bacterial)
Enzyme No.: EC 2.1.1.- (DNA modification methylase NgoBI); EC 2.1.1.73 (Site-Specific DNA Methyltransferase (Cytosine-Specific))
Record Date Created: 19971202
Record Date Completed: 19971202

12/9/7
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

Molecular Sequence Databank No.: GENBANK/U46781
CAS Registry No.: 0 (DNA Transposable Elements); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA Modification Methylases); EC 2.1.1.- (EcoR124 modification methylase type I); EC 2.1.1.72 (Site-Specific DNA-Methyltransferase (Adenine-Specific)); EC 3.1.21.3 (Deoxyribonucleases, Type I Site-Specific)
Record Date Created: 19961231
Record Date Completed: 19961231

12/9/34
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

13980187 PMID: 9677289
pyrH-encoded UMP-kinase directly participates in pyrimidine-specific modulation of promoter activity in Escherichia coli.
Kholti A; Charlier D; Gigot D; Huysveld N; Roovers M; Glansdorff N
Laboratoire de Microbiologie, Universite Libre de Bruxelles, 1-av. E. Gryson, Brussels, B-1070, Belgium.
Journal of molecular biology (ENGLAND) Jul 24 1998, 280 (4) p571-82, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The carAB operon of the enterics Escherichia coli K-12 and **Salmonella** typhimurium LT2, encoding the sole carbamoylphosphate synthetase (CPSase) of these organisms, is transcribed from two promoters in tandem, carP1 upstream and carP2 downstream, repressed respectively by pyrimidines and arginine. We present evidence that the pyrH **gene** product (the hexameric UMP-kinase) directly participates in the pyrimidine-specific control of carP1 activity. Indeed, we have isolated in E. coli a particular type of pyrH **mutation** (pyrH41) that retains a quasi-normal UMP-kinase activity, but yet is impaired in the pyrimidine-specific repression of the P1 promoter of the carAB operon of E. coli and of S. typhimurium. Moreover, the pyrimidine-dependent inhibition of in vivo Dam **methylase modification** of adenine -106 upstream of the carP1 promoter is **altered** in this pyrH **mutant** . The recessive pyrH41 allele bears a single C-G to A-T transversion that converts alanine 94 into glutamic acid (A94E). Although overexpression of pyrH41 results in UMP-kinase levels far above that of a wild-type strain, pyrimidine-specific repression of the carAB operon is not restored under these conditions. Similarly, overexpression of the UMP-CMP-kinase **gene** of Dictyostelium discoideum in the pyrH41 **mutant** does not restore pyrimidine-mediated control of carP1 promoter activity, in spite of the elevated UMP-kinase activity measured in such transformants. These results indicate that besides its catalytic function in the de novo pyrimidine biosynthesis, E. coli UMP-kinase fulfils an additional, but previously unrecognized role in the regulation of the carAB operon. UMP-kinase might function as the real sensor of the internal pyrimidine nucleotide pool and act in concert with the integration host factor (IHF) and aminopeptidase A (PepA alias CarP and XerB) in the elaboration of the complex nucleoprotein structure required for pyrimidine-specific repression of carP1 promoter activity. Copyright 1998 Academic Press.
Tags: Support, Non-U.S. Gov't
Descriptors: *Bacterial Proteins--genetics--GE; *Escherichia coli--genetics--GE; *Nucleoside-Phosphate Kinase--genetics--GE; *Promoter Regions (Genetics)-- **genetics** --GE; Adenine--chemistry--CH; Bacterial Proteins--metabolism--ME; DNA Methylation; Escherichia coli--enzymology--EN; Gene Expression Regulation, Bacterial; Mutation--genetics--GE; Nucleoside-Phosphate Kinase--metabolism--ME; Pyrimidines--metabolism--ME; Salmonella typhimurium--genetics--GE; Trans-Activation (Genetics)
CAS Registry No.: 0 (Bacterial Proteins); 0 (Pyrimidines); 73-24-5 (Adenine)
Enzyme No.: EC 2.7.4.- (uridine monophosphate kinase); EC 2.7.4.4

The NlaIII restriction enzyme isolated from Neisseria lactamica recognizes the sequence 5'-CATG-3', cleaving after the G to generate a four base 3' overhang. The NlaIII methylase and a portion of the NlaIII endonuclease gene were cloned into E. coli by the methylase selection method, and the remaining portion of the NlaIII endonuclease gene was cloned by inverse PCR. The nucleotide sequence of the endonuclease gene and the methylase gene were determined. The NlaIII endonuclease **gene** is 693 bp, encoding a protein with predicted molecular weight of 26487. The NlaIII **methylase gene** was identical with that previously reported [Labbe, D., Joltke, H.J. and Lau, P.C. (1990) Cloning and characterization of two tandemly arranged DNA methyltransferse **genes** of **Neisseria** lactamica: an adenine-specific M.NlaIII and a cytosine-type **methylase** . Mol. Gen. **Genet** . 224, 101-110]. The endonuclease and **methylase genes** overlap by four bases and are transcribed in the same orientation. The endonuclease **gene** was cloned into an improved T7 vector, and a high level of NlaIII endonuclease expression was achieved in E. coli.

Descriptors: *DNA Modification Methylases--genetics--GE; *Deoxyribonucleases, Type II Site-Specific--genetics--GE; *Escherichia coli--genetics--GE; *Genes, Structural, Bacterial--genetics--GE; *Neisseria--genetics--GE; Amino Acid Sequence; Bacteriophage T7--genetics--GE; Base Sequence; Cloning, Molecular--methods--MT; Gene Expression Regulation, Bacterial; Genetic Vectors--genetics--GE; Molecular Sequence Data; Neisseria --enzymology--EN; Sequence Analysis, DNA

Molecular Sequence Databank No.: GENBANK/U59398

CAS Registry No.: 0 (Genetic Vectors)

Enzyme No.: EC 2.1.1.- (DNA Modification Methylases); EC 3.1.21.- (endodeoxyribonuclease NlaIII); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)

Record Date Created: 19970213

Record Date Completed: 19970213

12/9/2

DIALOG(R)File 155:MEDLINE(R)

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12694301 PMID: 7616563

Pyrimidine regulation of the Escherichia coli and Salmonella typhimurium carAB operons: CarP and integration host factor (IHF) modulate the methylation status of a GATC site present in the control region.

Charlier D; Gigot D; Huysveld N; Roovers M; Pierard A; Glansdorff N

Research Institute of the CERIA-COOVI, Brussels, Belgium.

Journal of molecular biology (ENGLAND) Jul 21 1995, 250 (4) p383-91, ISSN 0022-2836 Journal Code: 2985088R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

By measuring the protection against Dam methylase modification of a GATC sequence located 106 bp upstream of the startpoint of promoter P1 in the control region of the carAB operon (encoding carbamoylphosphate synthetase) we have obtained evidence for a direct correlation between the degree of in vivo occupancy of a specific regulatory target site and the repressibility of the P1 promoter by pyrimidine residues. A high uridine nucleotide pool as well as binding of the carP (alias xerB/pepA) gene product and of the integration host factor (IHF) to the carAB control region are prerequisites to observe this in vivo protection. Purified CarP binds in vitro to the carAB control region and protects against DNase I two approximately 25 bp long stretches, one of which is located just downstream of the **GATC** sequence. **Mutations** in this site strongly impair the pyrimidine regulation of the P1 promoter and the interference with Dam **methylase modification** . These processes are also strongly impaired in the absence of integration host factor and in **mutants** affected in the IHF site located some 200 bp upstream of this Dam **methylase modification** site. IHF therefore exerts at least part of its antagonistic effects on P1, i.e. increased expression in minimal medium but increased repression in the

presence of pyrimidine residues, indirectly by influencing the formation or the stability of a particular protein-DNA complex. Furthermore, we demonstrate that the distance separating the IHF and Dam **methylase** target sites is crucial for the in vivo protection and for pyrimidine-mediated regulation of the promoter expression. **Mutations** **altering** this distance result in severe reductions of the degree of in vivo protection and, concomitantly, of the repressibility by pyrimidine residues of promoter P1 activity in a way indicative of the formation of a complex nucleoprotein structure. Since neither IHF nor CarP require pyrimidine residues to bind to the carAB control region, at least not in vitro, it is tempting to suggest that IHF and CarP-induced bending and looping provide changes in DNA topology that are required for assembling a specific pyrimidine-dependent nucleoprotein complex that modulates P1 activity.

Tags: Support, Non-U.S. Gov't

Descriptors: *Aminopeptidases--metabolism--ME; *Bacterial Proteins --metabolism--ME; *Carbamoyl-Phosphate Synthase (Glutamine-Hydrolyzing) --genetics--GE; *Promoter Regions (Genetics); *Pyrimidines--metabolism--ME; *Site-Specific DNA-Methyltransferase (Adenine-Specific)--metabolism--ME; Adenine--metabolism--ME; Aminopeptidases--genetics--GE; Animals; Bacterial Proteins--genetics--GE; Base Sequence; DNA-Binding Proteins--genetics--GE; DNA-Binding Proteins--metabolism--ME; Deoxyribonuclease I--metabolism--ME; Escherichia coli--genetics--GE; Escherichia coli--metabolism--ME; Glutamyl Aminopeptidase; Integration Host Factors; Methylation; Molecular Sequence Data; Mutation; Operon--genetics--GE; Repressor Proteins--genetics--GE; Repressor Proteins--metabolism--ME; Salmonella typhimurium--genetics--GE; Salmonella typhimurium--metabolism--ME; Transcription, Genetic--genetics --GE; Uracil Nucleotides--genetics--GE; Uracil Nucleotides--metabolism--ME

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA-Binding Proteins); 0 (Integration Host Factors); 0 (Pyrimidines); 0 (Repressor Proteins); 0 (Uracil Nucleotides); 0 (integration host factor, Salmonella); 73-24-5 (Adenine)

Enzyme No.: EC 2.1.1.- (Dam methyltransferase); EC 2.1.1.72 (Site-Specific DNA-Methyltransferase (Adenine-Specific)); EC 3.1.21.1 (Deoxyribonuclease I); EC 3.4.11 (Aminopeptidases); EC 3.4.11.7 (Glutamyl Aminopeptidase); EC 6.3.5.5 (Carbamoyl-Phosphate Synthase (Glutamine-Hydrolyzing))

Record Date Created: 19950824

Record Date Completed: 19950824

12/9/3

DIALOG(R)File 155:MEDLINE(R)

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08970372 PMID: 1856224

The HgaI restriction-modification system contains two cytosine methylase genes responsible for modification of different DNA strands.

Sugisaki H; Yamamoto K; Takanami M

Institute for Chemical Research, Kyoto University, Japan.

Journal of biological chemistry (UNITED STATES) Jul 25 1991, 266 (21) p13952-7, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A DNA fragment of about 3.4 kilobase pairs that expressed the HgaI modification activity was cloned from the chromosomal DNA of Haemophilus gallinarum, and its nucleotide sequence was determined. Two open reading frames (ORF) which could code for structurally similar proteins were identified in the upstream and middle regions and a truncated ORF in the downstream region in the same orientation. When the respective ORFs were separately cloned, the clones carrying the upstream and middle ORFs both expressed the modification activity, indicating that the two genes are involved in modification of the HgaI restriction-modification system. In order to determine the sites of modification precisely, the respective genes were recloned into an expression vector, from which **gene** products

07870810 PMID: 2842672

A mutation **in the DNA adenine** methylase **gene (dam) of Salmonella typhimurium decreases susceptibility to 9-aminoacridine-induced frameshift mutagenesis.**

Ritchie L; Podger D M; Hall R M
CSIRO Division of Molecular Biology, North Ryde, NSW, Australia.

Mutation research (NETHERLANDS) Sep 1988, 194 (2) p131-41, ISSN 0027-5107 Journal Code: 0400763
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

A mutant of Salmonella typhimurium with a reduced response to mutation induction by 9-aminoacridine (9AA) has been isolated. The mutation (dam-2) is located in the DNA adenine methylase gene. The dam-2 mutant strain exhibits a level of sensitivity to 2-aminopurine (2AP) intermediate between that of the dam+ and the DNA adenine methylation-deficit dam-1 strain, and 2AP sensitivity was reversed by introduction of a mutH mutation or of the plasmid pMQ148 (which carries a functional Escherichia coli dam+ gene). However, the dam-2 strain is not grossly defective in DNA adenine methylase activity. Whole cell DNA appears full methylated at -GATC- sites. The levels of 9AA required to induce equivalent levels of frameshift mutagenesis in the dam-2 strain were approximately 2-fold higher than for the dam+ strain. Introduction of pMQ148 dam+ reduced the level of 9AA required for induction of frameshift mutations 4-fold in the dam-2 strain and 2-fold in the dam+ strain. The dam-2 mutation had no effect on the levels of ICR191 required for induction of frameshift mutations, but introduction of pMQ148 reduced the ICR191-induced mutagenesis 2-fold. The dam+/pMQ148, dam-2/pMQ148 and dam-1/pMQ148 strains showed identical dose-response curves for both 9AA and ICR191. These results are consistent with a slightly reduced (dam-2) or increased (pMQ148) rate of methylation at the replication fork. The 2AP sensitivity of the dam-2 strain cannot be simply explained. Furthermore, addition of methionine to the assay medium reverses the 2AP sensitivity of the dam-2 strain, but has no effect on 9AA mutagenesis.

Descriptors: *Aminacrine--pharmacology--PD; *Aminoacridines--pharmacology --PD; * **Genes** , Bacterial; * **Genes** , Structural; * **Methyltransferases** -- **genetics** --GE; * **Mutation** ; * **Salmonella typhimurium** -- **genetics** --GE; DNA Transposable Elements; Genotype; Microbial Sensitivity Tests; Salmonella typhimurium--drug effects--DE; Salmonella typhimurium--enzymology--EN; Site-Specific DNA- **Methyltransferase** (Adenine-Specific); Species Specificity
CAS Registry No.: 0 (Aminoacridines); 0 (DNA Transposable Elements); 90-45-9 (Aminacrine)
Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.1.1.72 (Site-Specific DNA-Methyltransferase (Adenine-Specific))
Record Date Created: 19881004
Record Date Completed: 19881004

12/9/47

DIALOG(R)File 155:MEDLINE(R)
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08512708 PMID: 2185134

Characterization of the genetic **determinants of SsoII-restriction endonuclease and** modification **methyltransferase.**

Karyagina A S; Lunin V G; Nikolskaya I I
Institute of Medical Enzymology, U.S.S.R. Academy of Medical Sciences, Moscow.

Gene (NETHERLANDS) Mar 1 1990, 87 (1) p113-8, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

03196343 PMID: 4937994

Mutants **of T2gt with** altered **DNA** methylase **activity: relation to restriction by prophage P1.**

Revel H R; Hattman S M

Virology (UNITED STATES) Aug 1971, 45 (2) p484-95, ISSN 0042-6822

Journal Code: 0110674

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Descriptors: *Bacteriophages; *Coliphages--enzymology--EN; * **Genetics ,** Microbial; *Methyltransferases--metabolism--ME; * **Mutation** ; * **Shigella dysenteriae** ; **Adenine** ; Carbon Isotopes; Cell-Free System; Chromosome Mapping; Coliphages--metabolism--ME; Coliphages--pathogenicity--PY; Crosses, **Genetic** ; DNA; DNA, Bacterial--metabolism--ME; DNA, Viral --metabolism--ME; Escherichia coli; Filtration; Glucosyltransferases --metabolism--ME; Guanine; Methylation; Micrococcus; Recombination, **Genetic** ; Trichloroacetic Acid; Tritium

CAS Registry No.: 0 (Carbon Isotopes); 0 (DNA, Bacterial); 0 (DNA, Viral); 10028-17-8 (Tritium); 73-24-5 (Adenine); 73-40-5 (Guanine); 76-03-9 (Trichloroacetic Acid); 9007-49-2 (DNA)

Enzyme No.: EC 2.1.1. (Methyltransferases); EC 2.4.1.- (Glucosyltransferases)

Record Date Created: 19711202

Record Date Completed: 19711202

06706353 PMID: 6097301

[Comparison of specific recognition sites of adenine **and cytosine DNA-**methylase **of** Yersinia **Pestis EV 76 C dam and dcm by Escherichia coli** methylases **]**

Sravnenie spetsifichnosti saitov uznavaniia **adeninovoi** i tsitozinovoi DNK-metilaz **Yersinia** pestis EV 76 C dam i dcm metilazami Escherichia coli.

Demidova G V; Goncharov E K; Tynianova V I

Biokhimiia (Moscow, Russia) (USSR) Oct 1984, 49 (10) p1594-7, ISSN 0320-9725 Journal Code: 0372667

Document type: Journal Article ; English Abstract

Languages: RUSSIAN

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Using enzymatic modelling of in vitro methylation of chromosome DNAs from **Yersinia** pestis EV 76, E. coli 834 and E. coli C600 RII by DNA **methylases** of Eco RII and Eco dam as well as of DNA hydrolysis of plasmid pBR 322 from the cells of Y. pestis EV 76, E. coli C600 and E. coli 834 by restrictases of Eco RII and Cfu I, it was found that cytosine DNA **methylase** from plague bacteria does not correspond to the type of RII **methylases** of E. coli. Adenyl DNA **methylase** is related to E. coli **methylases** type dam and **modifies** **adenine** in the nucleotide sequence of **GATC** .

Tags: Comparative Study; In Vitro

Descriptors: *DNA (Cytosine-5-)- **Methyltransferase** --metabolism--ME; *DNA, Bacterial--metabolism--ME; *Escherichia coli--enzymology--EN; * **Methyltransferases** --metabolism--ME; * **Yersinia** pestis--enzymology--EN; Base Sequence; DNA Restriction Enzymes; DNA, Bacterial-- **genetics** --GE; Escherichia coli-- **genetics** --GE; Hydrolysis; Methylation; **Methyltransferases** -- **genetics** --GE; Plasmids; Site-Specific DNA-**Methyltransferase** (**Adenine** -Specific); **Yersinia** pestis-- **genetics** --GE

CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids)

Enzyme No.: EC 2.1.1. (**Methyltransferases**); EC 2.1.1.37 (DNA (Cytosine-5-)- **Methyltransferase**); EC 2.1.1.72 (Site-Specific DNA-**Methyltransferase** (**Adenine** -Specific)); EC 3.1.21 (DNA Restriction Enzymes)

Record Date Created: 19850315

Record Date Completed: 19850315

08142983 PMID: 3074017

Cloning, purification and characterization of the M.NdeI methyltransferase from Neisseria **denitrificans.**

Silber K R; Polisson C; Rees P A; Benner J S
New England Biolabs, Beverly, MA 01915.

Gene (NETHERLANDS) Dec 25 1988, 74 (1) p43-4, ISSN 0378-1119
Journal Code: 7706761

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Descriptors: *Bacterial Proteins-- **genetics** --GE; * **Neisseria** -- **genetics** --GE; *Site-Specific DNA-Methyltransferase (**Adenine** -Specific)-- **genetics** --GE; Bacterial Proteins--isolation and purification--IP; Escherichia coli -- **genetics** --GE; **Neisseria** --enzymology--EN; Recombinant Proteins-- **genetics** --GE; Recombinant Proteins--isolation and purification--IP; Site-Specific DNA-Methyltransferase (**Adenine** -Specific)--isolation and purification--IP
CAS Registry No.: 0 (Bacterial Proteins); 0 (Recombinant Proteins)
Enzyme No.: EC 2.1.1.- (DNA **modification methylase** NdeI); EC 2.1.1.72 (Site-Specific DNA-Methyltransferase (**Adenine** -Specific))
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Tn1725 transposon mutagenesis **of 9-18 delta 7, an EcoRI** deletion **derivative of** Salmonella **dublin lane plasmid pSDL2.**

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A 37.5-kb derivative of the **Salmonella** dublin virulence plasmid pSDL2 was subjected to **mutagenesis** with the transposon Tn1725. Fifty-two **insertions** were mapped, and the **mutants** were tested for their ability to restore virulence to a plasmid-free strain of S. dublin. Twenty-nine of these **inserts** could not restore full virulence and thus define nine regions of the plasmid essential for virulence. **Deletion** of a 4.5-kb region by Bal31 nuclease resulted in a 33-kb derivative that maintained full virulence.
Tags: Support, Non-U.S. Gov't
Descriptors: *DNA Transposable Elements; * **Mutagenesis** ; *Plasmids; * **Salmonella** -- **genetics** --GE; Animals; Mice; Mice, Inbred BALB C; **Salmonella** --pathogenicity--PY; Site-Specific DNA-Methyltransferase (**Adenine** -Specific)--pharmacology--PD; Virulence
CAS Registry No.: 0 (DNA Transposable Elements); 0 (Plasmids)
Enzyme No.: EC 2.1.1.- (DNA **modification methylase** EcoRI); EC 2.1.1.72 (Site-Specific DNA-Methyltransferase (**Adenine** -Specific))
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